

The synthesis and biological evaluation of a library of  
autoinducer-antibiotic conjugates

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# 1 Declaration

This dissertation describes work carried out in the Department of Chemistry, University of Cambridge under the supervision of Professor David Spring, and in the Department of Biochemistry, University of Cambridge under the supervision of Dr Martin Welch. This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution, except those parts which were included in my CPGS dissertation. The dissertation does not exceed the word limit specified by the Physics and Chemistry Degree Committee.

Lois Overvoorde

7th of September 2018

## 2 Abstract

Microbial resistance to antibiotics is a serious global health threat, and the discovery of new, safe and effective antibiotics is required urgently. A new class of antibiotics, namely siderophore-antibiotic conjugates, has shown promise in initial studies. Siderophores are used by bacteria for iron uptake, and so attaching antibiotics to them allows the antibiotic to be carried across cell membranes. This study investigated conjugates designed using a similar approach, but using bacterial autoinducers instead of siderophores. Autoinducers are required for coordination of bacterial behaviours and are involved in the control of swarming, virulence factor production and biofilm formation.

The quorum sensing molecules produced by *Pseudomonas aeruginosa* were chosen for investigation as *P. aeruginosa* is a significant human pathogen which displays high resistance to many antibiotics and uses quorum sensing to coordinate its group behaviours. Ciprofloxacin and trimethoprim were chosen as the antibiotic partners. Ciprofloxacin is commonly used against *P. aeruginosa* but resistance to it is developing, whereas *P. aeruginosa* is inherently resistant to trimethoprim. It was hypothesised that the autoinducers would aid retention of the antibiotics in cells, hence increasing or restoring activity.

An initial library was synthesised in two halves which were coupled together using a copper(I)-catalysed azide-alkyne cycloaddition. The autoinducers were functionalised with azide groups and the antibiotics (specifically ciprofloxacin and trimethoprim) were functionalised with alkynes. Two cleavable alkynyl ciprofloxacin derivatives were also included.

A second set of compounds, namely homoserine lactone analogue-ciprofloxacin conjugates were then synthesised, building on the one known report of a conjugate of a quorum sensing modulator and an antibiotic.

The most active conjugate found was a cleavable conjugate of homocysteine thiolactone (a homoserine lactone analogue) and ciprofloxacin. This compound showed enhanced antibacterial activity against *P. aeruginosa* compared to ciprofloxacin, and *P. aeruginosa* may develop less resistance towards it.

### 3 Acknowledgements

I would like to thank David Spring for the opportunity to work on this very interesting project. I would also like to thank Jamie Stokes for helping me get settled into the lab, James Hodgkinson for showing me the biological techniques I will need soon, and Felin Nie and Terrence Kwan for help with the LCMS. Thanks also to Matt Pond, Melvyn Orriss and Nic Davies for help with equipment and glassware and to Jill Vaughan for proof-reading. Most importantly, I would like to thank Yssy Baker for proof-reading, help, support, advice and encouragement. Finally, I would like to thank everyone in the Spring group for their help, advice and delightful company.

## 4 Nomenclature

$m$	Mass
$v$	Volume
$J$	Coupling constant in Hz
$m/z$	Mass to charge ratio in Daltons
$R_f$	Retention factor
Ac	Acetate
AIP	Autoinducing peptide
aq.	Aqueous
atm	Atmosphere(s)
BHL	Butyryl homoserine lactone = C <sub>4</sub> -HSL
Boc	<i>tert</i> -Butyloxycarbonyl
Bu	Butyl
Cip	Ciprofloxacin
conc.	Concentrated
COSY	Correlation spectroscopy
d	Day(s)
Da	Daltons
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	Equivalents
ESI	Electrospray ionization
Et	Ethyl
FT	Fourier transform
h	Hour(s)
HCTL	Homocysteine thiolactone



HHQ 2-Heptylquinolin-4(1H)-one  
 HMBC Heteronuclear multiple-bond correlation spectroscopy  
 HMQC Heteronuclear multiple-quantum correlation spectroscopy  
 HOBt 1-Hydroxybenzotriazole  
 HPLC High-performance liquid chromatography  
 HRMS High resolution mass spectroscopy  
 HSL Homoserine lactone  
 Hz Hertz  
 IR Infrared  
 LB Lysogeny broth  
 LCMS Liquid chromatography mass spectroscopy  
 LCT Liquid chromatography time-of-flight  
 lit. Literature value  
 M Molar  
 m.p. Melting point  
 Me Methyl  
 MIC Minimum inhibitory concentration  
 min Minute(s)  
 mol Mole(s)  
 Ms Methanesulfonyl  
 NMP *N*-Methyl-2-pyrrolidone  
 NMR Nuclear magnetic resonance  
 OD Optical density  
 OdDHL *N*-(3-Oxododecanoyl)-homoserine lactone = 3-oxo-C<sub>12</sub>-HSL  
 P.E. Petroleum ether  
 PAI-1 *Pseudomonas* autoinducer 1 = 3-oxo-C<sub>12</sub>-HSL  
 PAI-2 *Pseudomonas* autoinducer 2 = C<sub>4</sub>-HSL  
 Pd/C Palladium on carbon  
 PQS *Pseudomonas* Quinolone Signal  
 Q-TOF Quadrupole time-of-flight  
 r.t. Room temperature  
 s Second(s)

SAM	<i>S</i> -adenosyl-L-methionine
SAR	Structure activity relationship
sat.	Saturated
SD	Standard deviation
spp.	Species
TBAF	Tetrabutylammonium fluoride
TBDMS	<i>tert</i> -Butyldimethylsilyl
TEA	Triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THPTA	Tris(3-hydroxypropyltriazolymethyl)amine
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts	<i>para</i> -Toluenesulfonyl
UV	Ultraviolet

## 5 Introduction

### 5.1 Antibiotic resistance

Antibiotics add, on average, twenty years to a person's life.<sup>1</sup> However, antibiotic resistance is increasing alarmingly and is now recognised as a major threat to global health.<sup>1,2</sup> Antibiotic discovery had its heyday in the 1940s to 60s, which saw the discovery of many new classes of antibiotic. Since then, the rate of discovery of new classes has slowed, and resistance to existing treatments has increased.

The story of how Alexander Fleming discovered penicillin by accidentally allowing a Petri dish containing *Staphylococcus aureus* to become contaminated with *Penicillium* mould whilst he was on holiday in Suffolk<sup>1</sup> is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,<sup>3</sup> but it was not until 1943 that the drug was mass produced thanks to the research of Ernst Chain and Howard Florey. Unfortunately, bacterial resistance to penicillin was being found in hospitals by the late 1940s.<sup>4,5</sup> This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see Table 1) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories:<sup>1,6</sup>

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of  $\beta$ -lactam antibiotics such as penicillin by  $\beta$ -lactamase enzymes.
2. The bacterium may produce a membrane, cell wall or biofilm which does not allow the drug to pass through. For example, biofilm formation may allow bacterial resistance to antibiotics to increase 1000-fold compared with bacteria in suspension culture.<sup>7</sup>
3. The bacterium may pump antibacterial molecules out of its cell membrane using efflux pumps, for example the MexAB and MexXY pumps used by *Pseudomonas aeruginosa*.<sup>8</sup>
4. Mutations may cause the target of the antibacterial molecule to alter such that the molecule no longer effectively binds the target, for example the alteration of penicillin binding proteins which are involved in the final stages of peptidoglycan biosynthesis in the cell walls of MRSA and other penicillin-resistant bacteria.<sup>9</sup>
5. The bacterium may switch to using a metabolic pathway which does not involve the target of the antibacterial molecule, for example sulfonamide resistance may be achieved by taking in folic acid from the environment rather than synthesising it using *para*-aminobenzoic acid - a process which is blocked by sulfonamides.<sup>10</sup>

Antibiotic	Introduction	Resistance
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Trimethoprim	1962	1972
Cephalosporins	1960s	late 1960s
Ciprofloxacin	1987	1988
Linezolid	2000	1997*
Daptomycin	2003	2005

Table 1: A timeline of when various antibiotics were first introduced and when resistance to them first appeared.<sup>11–16</sup> \*Resistance was seen during a compassionate-use program before the drug was widely released.<sup>15</sup>

The current pipeline of new antibiotics is widely thought to be worryingly inadequate.<sup>17–19</sup> Significant changes in how we use the antibiotics we already have, as well as investments in the discovery of new ones, are required. Antibiotics currently in late-stage clinical trials nearly all rely on non-novel mechanisms of action,<sup>17</sup> and so it is almost inevitable that resistance to them will develop quickly, as it has done for their predecessors.

There is therefore increasing interest in treatments which would not easily provoke the development of resistance.<sup>20</sup> These treatments often target bacterial virulence rather than killing bacteria outright, hence decreasing selection pressure for resistance.<sup>11</sup> One obvious target is toxin production, for example, an LpxC inhibitor was shown to prevent lethal *Acinetobacter baumannii* infection in mice, despite being inactive against the bacterium *in vitro*.<sup>21</sup> This was due to inhibition of lipopolysaccharide shedding, and hence reduced inflammation in the host. Co-ordination of virulence has also been targeted, for example, analogues of *P. aeruginosa* homoserine lactone (HSL) autoinducers (see 5.3.1) inhibit the production of virulence factors and increase the survival time of mice in a lethal *P. aeruginosa* lung infection model.<sup>11</sup>

A second strategy in novel antibiotic discovery is to enhance or restore activity of a known antibiotic by lessening or avoiding a resistance mechanism. For example, antibiotics are often excluded from cells due to membrane impermeability or efflux. This may be overcome by attaching the antibiotic ‘warhead’ to a molecule which the cell imports. The most well known example of this strategy is antibody-drug conjugates<sup>22</sup> in the treatment of cancer, but progress has also been made against bacteria. In particular, siderophore-antibiotic conjugates (see 5.2) have been investigated in the hope of hijacking bacterial uptake mechanisms to import antibiotics,<sup>23</sup> and the autoinducer-antibiotic conjugates in this study may gain activity by avoiding efflux pumps (see 5.3). These conjugates may have competing mechanisms of action: either the antibiotic accumulates in the cell to a greater extent and acts by its usual mechanism, or an important bacterial system must be disrupted to avoid accumulation of the antibiotic, hence leading to decreased fitness and/or loss of virulence.

## 5.2 Siderophore-antibiotic conjugates

Siderophore-antibiotic conjugates have been receiving attention in recent years as a way to enhance the uptake of known antibiotics.<sup>23</sup> This section will discuss the role of siderophores, sideromycins (natural siderophore-antibiotic conjugates), and the synthetic siderophore-antibiotic conjugates inspired by them. Many of the ob-

servations made about these molecules could be relevant to the autoinducer-antibiotic conjugates synthesised in this study.

### 5.2.1 Siderophores

Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of ‘iron mining’.<sup>24</sup> Soluble iron is often scarce but it is crucial for many cellular processes including respiration and DNA synthesis. Siderophores are synthesised by the microorganisms and secreted into the extracellular environment where they bind to  $\text{Fe}^{3+}$ , often with exceptionally high affinities. The iron-bound siderophores are then brought back into the cell by active transport and the iron is released, either by reduction of the  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  or by enzymatic degradation of the siderophore. Siderophores have a wide range of structures (see Figure 2 and Figure 1), possibly so one species can avoid its siderophores being taken up by another species.<sup>25</sup>

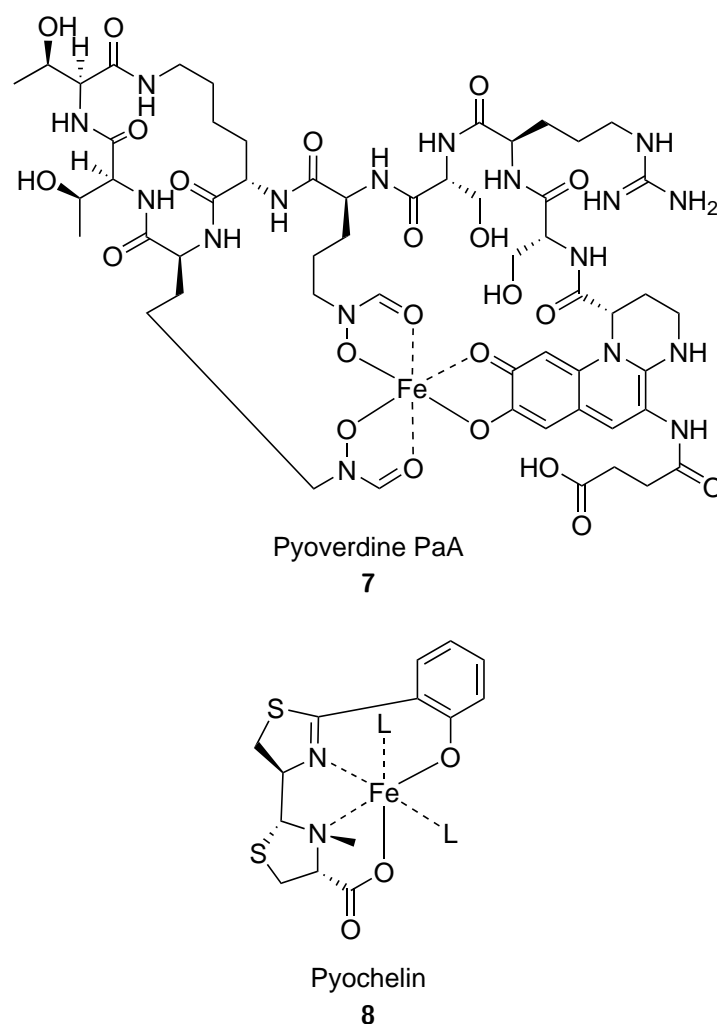


Figure 1: Iron-siderophore complexes: pyoverdine PaA **7**<sup>26, 27</sup> (*P. aeruginosa*, PAO1 strain) and pyochelin **8**<sup>28, 29</sup> (*P. aeruginosa*). Note that pyochelin **8** is a tetradentate ligand, hence the iron ion has two sites which can bind other ligands.

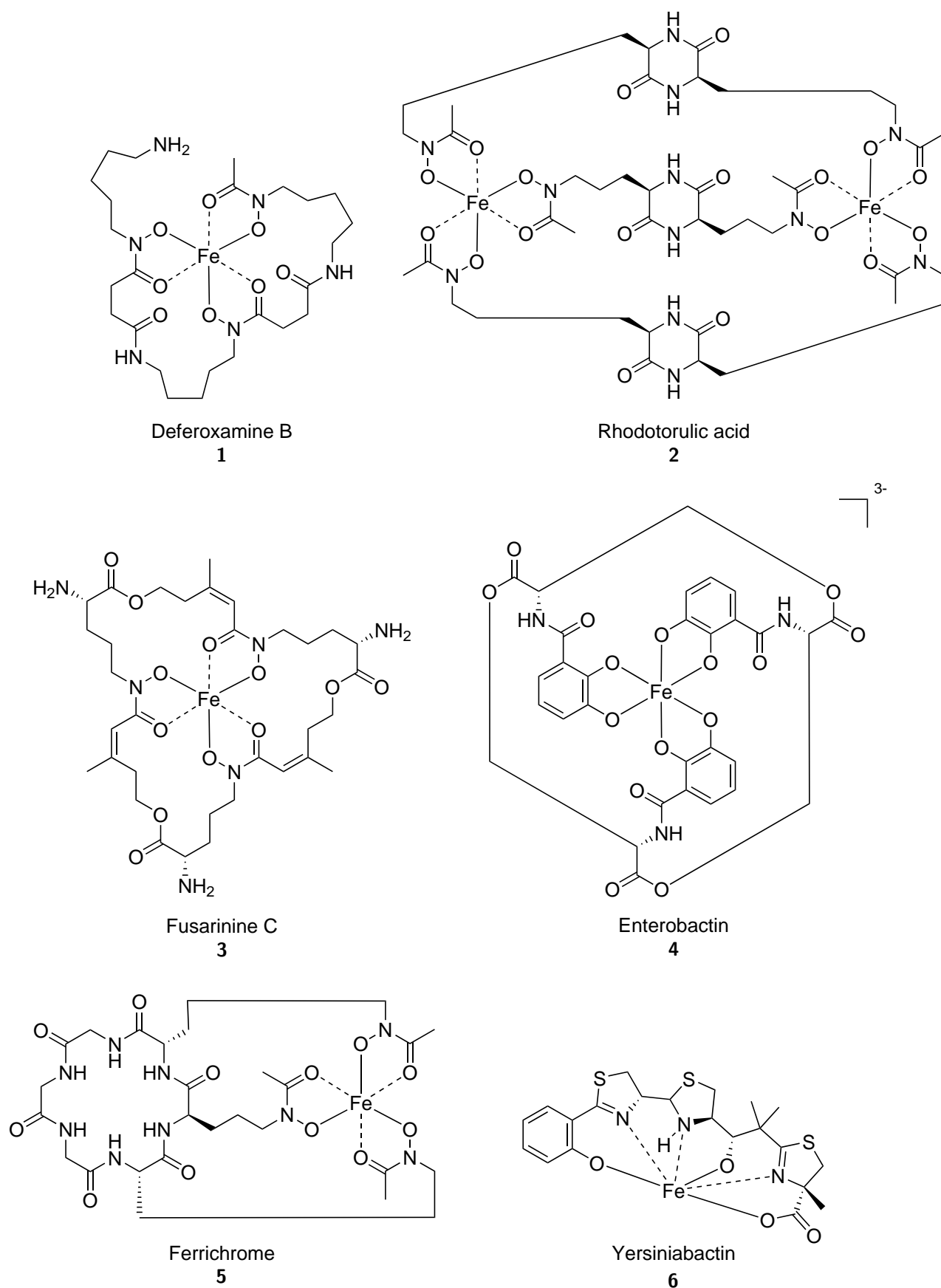


Figure 2: Iron-siderophore complexes: Deferoxamine B **1**<sup>26</sup> (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid **2**<sup>30</sup> (*Rhodotorula pilimanae*), fusarinine C **3**<sup>31</sup> (*Fusarium roseum*), enterobactin **4**<sup>26</sup> (*Escherichia coli* and enteric bacteria), ferrichrome **5**<sup>32</sup> (*Ustilago sphaerogena*, *U. maydis*, *Aspergillus niger*, *A. quadricinctus*, *A. duricaulis* and *Penicillium resticolosum*), yersiniabactin **6**<sup>26</sup> (*Yersinia pestis*).

### 5.2.2 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins<sup>23</sup> (see Figure 3). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds.

For example, albomycin **9** (see Figure 3) is a sideromycin produced by *Actinomyces subbropicus* and *Streptomyces griseus*<sup>33,34</sup> which has been used to treat infections caused by various bacteria including *Yersinia enterocolitica* and *Streptococcus pneumoniae* in mice and humans.<sup>35,36</sup> Albomycin **9** contains a siderophore coupled to a nucleoside antibiotic via a peptide linker. The siderophore section is structurally similar to ferrichrome **5** (see Figure 2), a siderophore produced by various fungi, but also taken up by bacteria including *Escherichia coli*, *Salmonella typhimurium* and *P. aeruginosa*.<sup>32,37</sup> It has been shown that because of the structural similarity to ferrichrome **5**, *E. coli* will also take up albomycin **9**.<sup>33</sup> The linker is hydrolysed in the cytoplasm of the *E. coli*, releasing the active nucleoside antibiotic. This leads to 500-fold concentration of the antibiotic within the *E. coli* cells, enough to have significant effect on growth.

The success of albomycin<sup>35</sup> and other sideromycins such as salmycin A<sup>24,38,39</sup> and ferrimycin A1<sup>40,41</sup> has served as encouragement to many researchers to explore synthetic siderophore-antibiotic conjugates, which will be discussed in the next section.

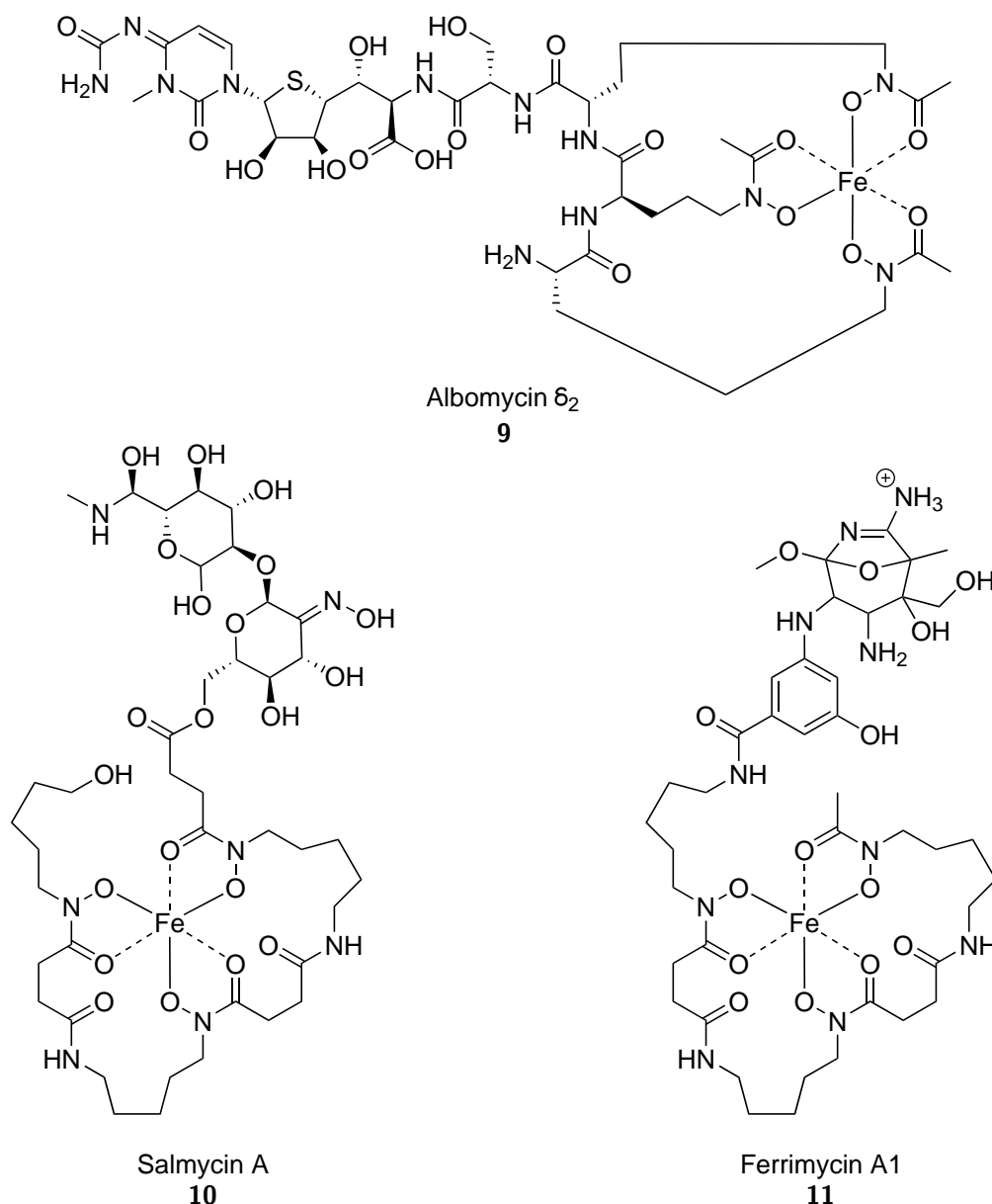


Figure 3: Iron-sideromycin complexes: Albomycin **9**<sup>24,42</sup> (*Actinomyces subtropicus* and *Streptomyces griseus*), salmycin A<sup>24,38,39</sup> (*Streptomyces violaceus*) and ferrimycin<sup>24</sup> (*Streptomyces griseoflavus*).

### 5.2.3 Synthetic siderophore-antibiotic conjugates

Sideromycins served as inspiration for the design, synthesis and biological evaluation of a wide range of synthetic siderophore-antibiotic conjugates.<sup>23</sup> Antibiotics used include  $\beta$ -lactams,<sup>43–45</sup> nucleosides,<sup>46</sup> glycopeptides<sup>47</sup> and macrolides.<sup>48</sup> Sideromycin-fluoroquinolone conjugates have also been studied by several groups,<sup>49–51</sup> including conjugates with linkers which can be cleaved<sup>50,51</sup> in a similar manner to albomycin.<sup>33</sup> Some of these showed comparable activity to the parent antibiotic, but it is not clear whether attachment of the siderophore improved uptake or whether the conjugates acted as classical prodrugs.

$\beta$ -lactam-sideromycin conjugates have been more widely investigated and show good activity *in vitro*. However, resistance can evolve by loss of the TonB transporter or of the relevant siderophore receptor, e.g. Cir and Fiu for catecholate siderophores or FhuA for hydroxamate siderophores.<sup>23</sup> Recently a conjugate (Ent-Amp **12**, see Figure 4) of enterobactin and ampicillin joined using a copper(I)-catalyzed azide-alkyne cycloaddition has been shown to have increased activity against pathogenic *E. coli* when compared to native ampicillin.<sup>52</sup> Other



work has focused on monocyclic  $\beta$ -lactams, for example pirazmonam **13** and U-78608 **14**, which show high potency against Gram-negative bacteria including *P. aeruginosa*,<sup>53,54</sup> Monocyclic  $\beta$ -lactams are generally fairly stable to  $\beta$ -lactamase activity, which is an advantage compared with many bicyclic  $\beta$ -lactams.

Three siderophore-antibiotic conjugates are reported as being in clinical trials:<sup>55</sup> MC-1 **15**,<sup>56</sup> BAL30072 **16**<sup>23</sup> (see Figure 4) and cefiderocol **17**.<sup>57,58</sup>

MC-1 **15** is reported as being ‘in clinical phases of development’,<sup>55</sup> but no reports of studies in humans could be found. However, experiments in mice have been promising.<sup>56</sup> BAL30072 **16** is a siderophore- $\beta$ -lactam conjugate which showed initial promise as it is a poor substrate for  $\beta$ -lactamases, and resistance due to loss of transport proteins is infrequent.<sup>23</sup> However, it is unclear whether it will progress further in trials as it causes liver toxicity.<sup>59</sup> Cefiderocol **17** is a cephalosporin-catechol conjugate in phase 1 trials. Recent results indicate that ‘single and 35 multiple intravenous doses of cefiderocol at up to 2000 mg were well tolerated in healthy 36 subjects’.<sup>58</sup>

These examples show that siderophore-antibiotic conjugates are a promising strategy to deliver antibiotics across bacterial membranes, but it is worth noting that conjugation to a siderophore may lead to loss of activity, or resistance may be acquired by loss of transport proteins. Encouragingly though, albomycin **9**-resistant mutants have been shown to be less virulent,<sup>36</sup> indicating that bacteria may lose out either by susceptibility to the antibiotic or by loss of fitness due to decreased iron transport.

Building on these positive examples, it is hoped that the strategy of conjugating a molecule which is important for virulence<sup>60</sup> with an antibiotic can be extended to conjugates of autoinducers and antibiotics in a similar ‘Trojan horse’ approach.

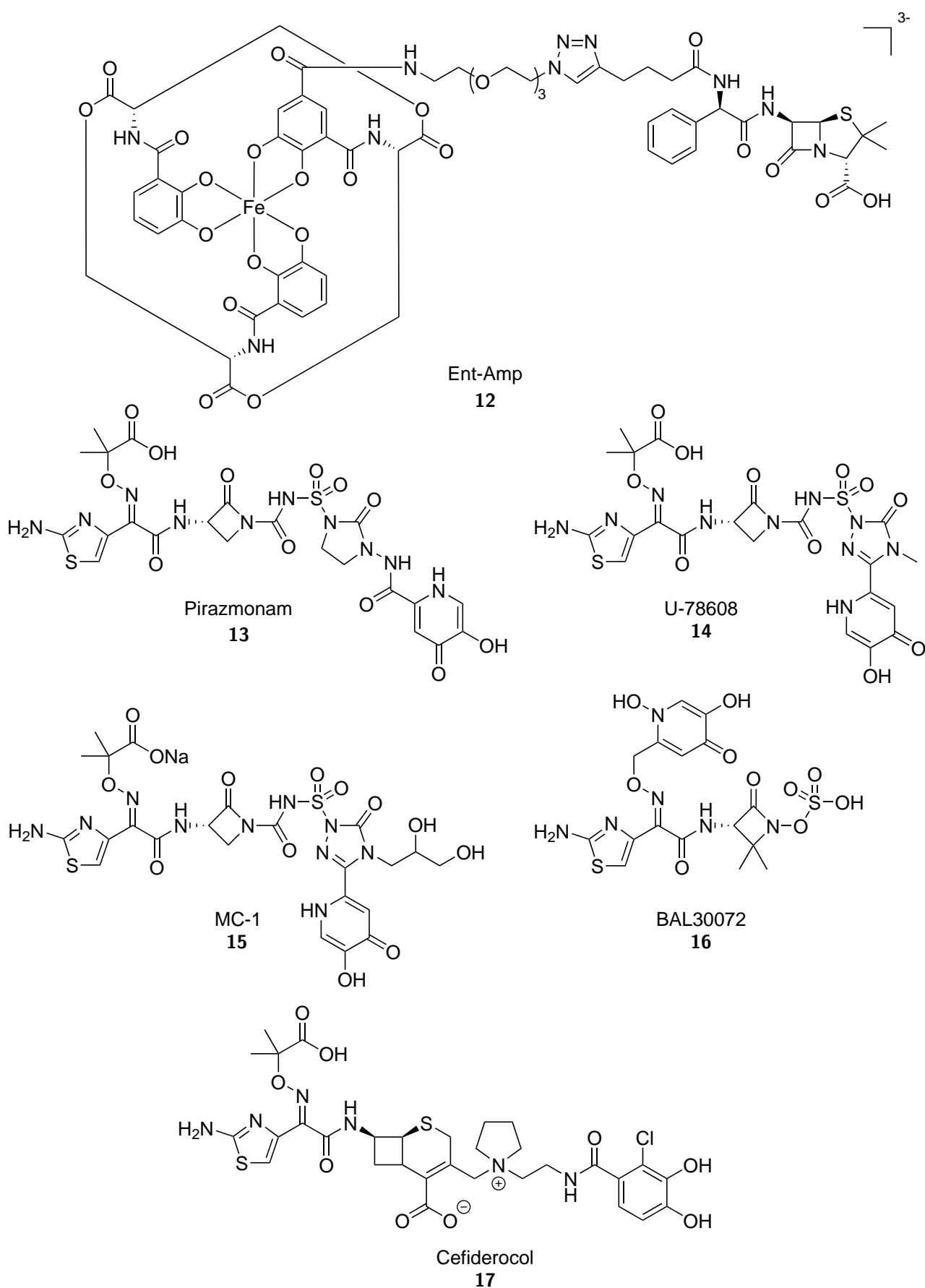


Figure 4: Examples of siderophore-antibiotic conjugates: Ent-Amp **12**,<sup>52</sup> pirazmonam **13**,<sup>53,54</sup> U-78608 **14**,<sup>53,54</sup> MC-1 **15**,<sup>56</sup> BAL30072 **16**<sup>23</sup> and cefiderocol **17**.<sup>57,58</sup>

### 5.3 Autoinducer-antibiotic conjugates

This study extends the conjugation strategy discussed above by creating autoinducer-antibiotic conjugates. It was hypothesised that attaching an autoinducer to a known antibiotic could lead to increased cellular retention of the antibiotic, and could potentially restore function against resistant strains. This is thought to be the first large study of autoinducer-antibiotic conjugates, with only one such molecule having been reported previously<sup>61</sup> (see 5.3.8). This section begins by introducing the concept of quorum sensing, followed by discussion of the autoinducers and antibiotics used in this study and the mechanisms of their efflux from *P. aeruginosa* cells, and how these mechanisms could be exploited by conjugates.

#### 5.3.1 Quorum sensing

A quorum is defined as ‘A fixed minimum number of members of an assembly or society that must be present at any of its meetings to make the proceedings of that meeting valid.’<sup>62</sup> A similar concept is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum concentration of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors, swarming and biofilm formation.<sup>63</sup> It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium. The process by which bacteria determine the concentration of similar bacteria in their vicinity, and act on that information, is known as quorum sensing.

Quorum sensing has been observed in many species of bacteria, including *Vibrio fischeri*, *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *S. aureus*, *V. harveyi*, *Escherichia coli*, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica*, *Aeromonas spp.* and *Acinetobacter spp.*<sup>63–72</sup> Many of these bacteria are significant causes of disease and death in humans, for example, in a typical year in the U.S. *P. aeruginosa* causes 6,700 multidrug-resistant infections and 440 deaths, methicillin-resistant *S. aureus* causes 80,500 severe infections and 11,300 deaths and non-typhoidal *Salmonella* causes 1.2 million illnesses, 23,000 hospitalisations and 450 deaths.<sup>2</sup>

##### 5.3.1.1 *Vibrio fischeri*

The first example of quorum sensing was discovered in *V. fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid, *Euprymna scolopes*<sup>63,71,72</sup> (see Figure 5). This bacterium receives amino acids<sup>73,74</sup> from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself.<sup>75</sup>

If a low population of *V. fischeri* were present in the photophore, the light that the bacteria could produce would be insufficient to provide counterillumination. Therefore, the bacteria conserve resources by not producing light. However, if there is a high population of *V. fischeri* it is useful for them all to produce light, as this incentivises the squid to provide them with nutrients.



Figure 5: ‘Euprymna scolopes, South shore of Oahu, Hawaii’ by Jamie Foster. Licensed under CC BY-SA 3.0 via Commons.

*V. fischeri* uses the LuxR-LuxI system to sense cell density. This system is seen as a paradigm of quorum sensing, and a simplified explanation of it is presented to show typical features of such a system (see Figure 6).

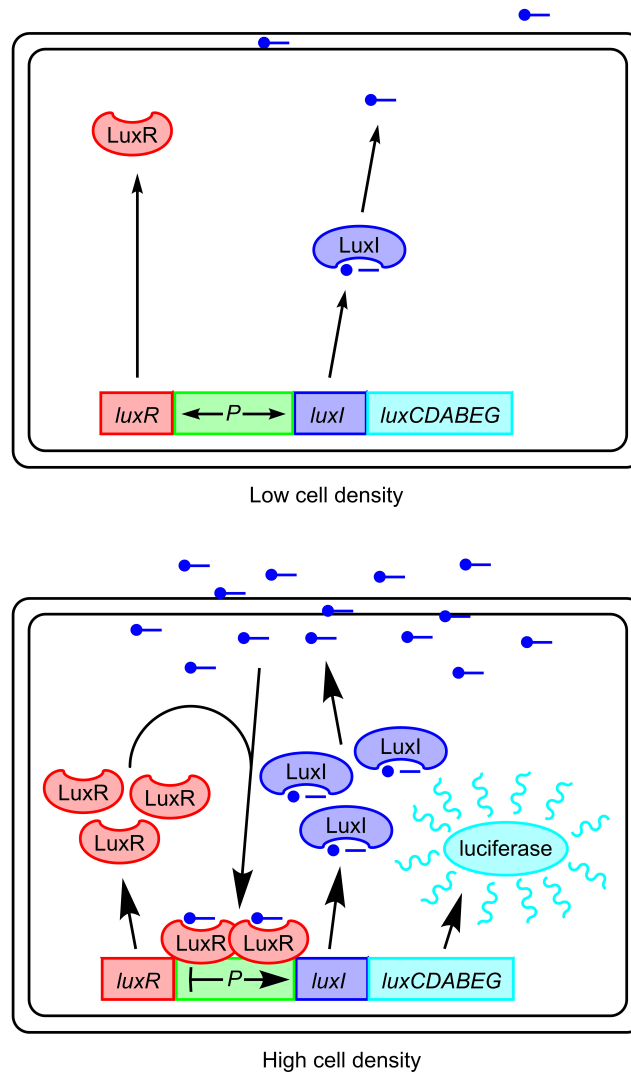


Figure 6: The LuxR-LuxI quorum sensing system in *V. fischeri*.

*V. fischeri* senses cell concentration by the detection of 3-oxo-C<sub>6</sub>-HSL **18**<sup>76</sup> (see Figure 7), a freely diffusible<sup>77</sup> molecule which is synthesised by LuxI<sup>78,79</sup> and secreted by all *V. fischeri* cells<sup>80</sup> at a low basal level.<sup>63</sup> When

the bacterial population density, and hence the concentration of 3-oxo-C<sub>6</sub>-HSL **18**, reaches a threshold, 3-oxo-C<sub>6</sub>-HSL **18** binds to LuxR,<sup>81–83</sup> a receptor which is also synthesised at a low basal level.

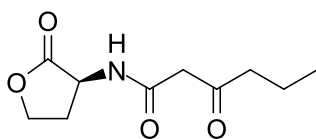


Figure 7: 3-oxo-C<sub>6</sub>-HSL **18**.

The LuxR complex binds to the *lux* operator, upregulating production of LuxI and hence 3-oxo-C<sub>6</sub>-HSL **18**, and luciferase enzymes and hence blue-green light.<sup>84–86</sup> Production of more 3-oxo-C<sub>6</sub>-HSL **18** enables a positive feedback loop, reinforcing the effect of high population density on 3-oxo-C<sub>6</sub>-HSL **18** concentration and hence light production. This is the reason that 3-oxo-C<sub>6</sub>-HSL **18** is known as an autoinducer.

The system also contains a negatively feedback loop to avoid excessive expression of proteins: at high concentrations of 3-oxo-C<sub>6</sub>-HSL **18** production of LuxR is inhibited.<sup>87</sup> Such balancing effects, as well as interactions with other quorum sensing and metabolic systems, are seen in many bacteria.<sup>63,88</sup>

### 5.3.1.2 *Pseudomonas aeruginosa*

Another well-studied example of quorum sensing is in *P. aeruginosa*.<sup>88–90</sup> *P. aeruginosa* is a Gram-negative opportunistic pathogen which typically infects immunocompromised individuals such as those with cystic fibrosis, neutropenia and AIDS. It can infect the pulmonary and urinary tracts as well being the most frequent cause of burn wound infections and the most frequent coloniser of medical devices such as catheters.<sup>91</sup> Multidrug-resistant *P. aeruginosa* is classified as a ‘serious threat’ by the United States Centers for Disease Control and Prevention<sup>2</sup> and carbapenem-resistant *P. aeruginosa* is classified as ‘priority 1: critical’ by the World Health Organisation.<sup>18</sup>

*P. aeruginosa* has a low susceptibility to many antibiotics and readily acquires antibiotic resistance by mutation or horizontal gene transfer.<sup>92</sup> It is difficult for antibiotics to cross into cells due to low cell membrane permeability,<sup>93</sup> and they are pumped out again by its multiple chromosomally encoded multidrug efflux pumps.<sup>8</sup> *P. aeruginosa* also forms biofilms: colonies of microbes held together and protected by a matrix containing polysaccharides, proteins and DNA. Bacteria are usually studied in the planktonic state, where they are present as free-floating cells which are often more metabolically active. However, the study of bacteria in biofilms is important due to their importance in chronic infections and the colonisation of implanted medical devices.<sup>7,94</sup> *P. aeruginosa* biofilms are more resistant to many drugs including ciprofloxacin **24** and trimethoprim **25** compared with planktonic cells.<sup>95,96</sup> This high level of antibiotic resistance makes *P. aeruginosa* an important target for drug discovery.

Quorum sensing in *P. aeruginosa* involves a complex interplay of five signalling molecules (see Figure 8) and various proteins (see Figure 9).<sup>88–90</sup> These can be broken down into three main, interacting systems: Las, Rhl and Pqs.

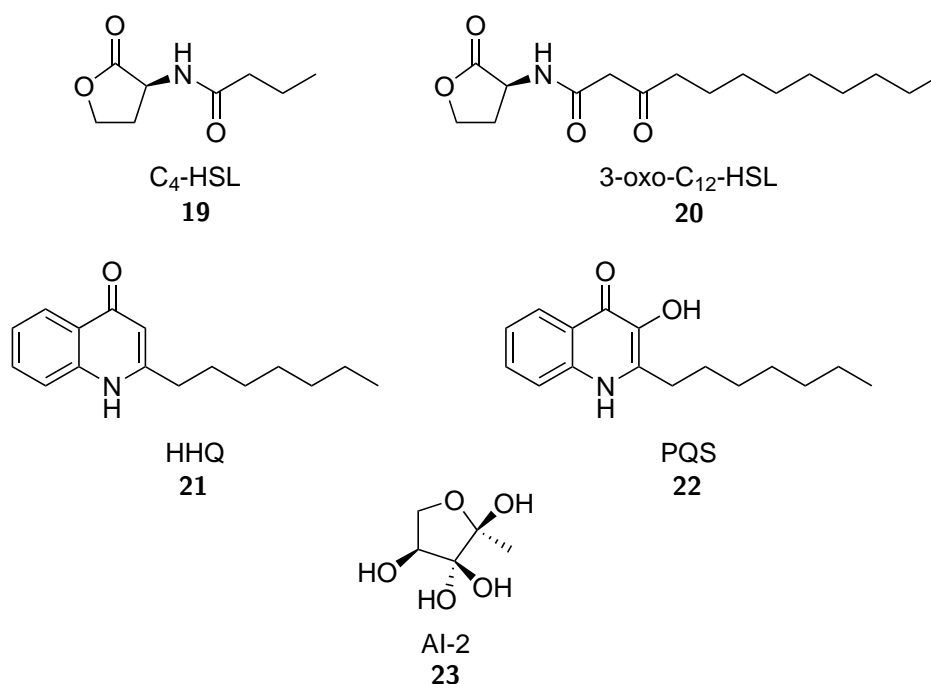


Figure 8: *P. aeruginosa* autoinducers.

In the Las system, LasI<sup>97</sup> synthesises the 3-oxo-C<sub>12</sub>-HSL **20**<sup>98</sup> autoinducer. 3-oxo-C<sub>12</sub>-HSL **20** binds LasR,<sup>99</sup> and this complex upregulates the production of LasI<sup>100</sup> (thus causing autoinduction) as well as alkaline protease,<sup>101</sup> elastase,<sup>99</sup> exotoxin A,<sup>101</sup> HCN<sup>102</sup> and LasA protease.<sup>103</sup> The LasR complex is also important in late-stage biofilm formation,<sup>68</sup> and upregulates the Rhl<sup>104</sup> and Pqs systems.<sup>105, 106</sup>

In the Rhl system, RhlI<sup>107</sup> synthesises the C<sub>4</sub>-HSL **19**<sup>108</sup> autoinducer. C<sub>4</sub>-HSL **19** binds RhlR,<sup>109</sup> and this complex upregulates the production of RhlI<sup>100</sup> (again causing autoinduction), alkaline protease,<sup>110</sup> elastase,<sup>107</sup> haemolysin,<sup>110</sup> HCN,<sup>102, 110</sup> LasA protease,<sup>107</sup> LecA,<sup>111</sup> pyocyanin<sup>107, 110</sup> and rhamnolipids.<sup>107</sup> The RhlR complex also downregulates the Pqs system.<sup>106, 112</sup> The Rhl system is controlled by both the Las and Pqs systems, as production of both RhlR and RhlI is upregulated by the LasR complex<sup>104</sup> and production of both RhlR is upregulated by the PqsR complex.<sup>113</sup>

In the Pqs system, the main autoinducer, PQS **22**,<sup>114</sup> is synthesised by multiple enzymes: PhnAB,<sup>115</sup> PqsA, PqsBC, PqsD<sup>116, 117</sup> and PqsE<sup>118, 119</sup> produce the precursor HHQ **21**, and PqsH converts HHQ **21** to PQS **22**. PQS **22**<sup>106</sup> or HHQ **21** binds PqsR,<sup>120</sup> and either complex can upregulate the synthesis of HHQ **21** causing autoinduction. The PqsR-PQS complex upregulates the production of chitinase,<sup>121</sup> elastase,<sup>114</sup> HCN,<sup>121</sup> LecA,<sup>122</sup> pyocyanin<sup>105, 123</sup> and pyoverdine,<sup>123</sup> as well as increasing biofilm production<sup>122</sup> and vesicle formation.<sup>124</sup> The PqsR-PQS complex also upregulates production of RhlR, so the Pqs system has control over the Rhl system.<sup>113</sup> The Pqs system is controlled by both the Las and Rhl systems, as production of PqsR<sup>106</sup> and PqsH<sup>105</sup> is upregulated by the LasR complex and production of PqsA, PqsBC, PqsD, PqsE<sup>112</sup> and PqsR<sup>106</sup> is downregulated by the RhlR complex.



a library of autoinducer-antibiotic conjugates was synthesised to test the hypothesis that the importance of autoinducers in harmful cellular behaviours could lead to increased activity of the conjugates (see 5.3).

The *P. aeruginosa* autoinducers (see Figure 8) were chosen for use in this study as *P. aeruginosa* is a significant human pathogen which shows high antibiotic resistance and utilises quorum sensing to coordinate pathogenic behaviours (see 5.3.1.2). Specifically, C<sub>4</sub>-HSL **19**, HHQ **21** and PQS **22** derivatives were chosen as they were considered to be the most synthetically tractable.

### 5.3.3 Autoinducer efflux

Autoinducers must be exported from the cell in order to be used for intercellular communication, and the five known *P. aeruginosa* autoinducers are exported by various different transport mechanisms. The mechanism is not well known for HHQ **21** or AI-2 **23**, but it is known that PQS **22** is exported in vesicles,<sup>129</sup> C<sub>4</sub>-HSL **19** passively diffuses in and out of cells,<sup>130</sup> and 3-oxo-C<sub>12</sub>-HSL **20** is taken up passively, accumulates in the cell membrane and is actively pumped out by efflux pumps. The difference in transport mechanism for C<sub>4</sub>-HSL **19** and 3-oxo-C<sub>12</sub>-HSL **20** is thought to be largely due to chain length rather than the 3-oxo modification, as a shorter-chain version, 3-oxo-C<sub>6</sub>-HSL **18** has been shown to be freely diffusible through *V. fischeri* membranes.<sup>77</sup>

3-oxo-C<sub>12</sub>-HSL **20** is exported primarily via the MexAB-OprM efflux system.<sup>8,131</sup> The increased removal of 3-oxo-C<sub>12</sub>-HSL **20** from the cell by upregulation of the MexAB-OprM system leads to decreased production of additional 3-oxo-C<sub>12</sub>-HSL **20** (as the positive feedback loop is disrupted, see 5.3.1.2), and hence decreased production of pyocyanin, elastase and casein protease. It is expected that MexAB-OprM upregulation would also disrupt biofilm formation as a decrease in 3-oxo-C<sub>12</sub>-HSL **20** levels would disrupt Las-mediated quorum sensing,<sup>132</sup> but no direct studies of this could be found.

### 5.3.4 Antibiotics

Ciprofloxacin **24** and trimethoprim **25** (see Figure 10) were chosen as the antibiotic sides of the conjugates.

Ciprofloxacin **24** is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections including *P. aeruginosa*.<sup>133,134</sup> Ciprofloxacin **24** inhibits DNA replication by binding to DNA gyrase and topoisomerase IV.<sup>135</sup>

Trimethoprim **25** (see Figure 10) is a dihydrofolate reductase inhibitor used primarily to treat bladder infections.<sup>136</sup> It is active against several significant human pathogens including *Streptococcus pneumoniae* and *Haemophilus influenzae*, but not against *P. aeruginosa*. It was primarily chosen in this study as it was considered easy to functionalise, but also to test the feasibility of creating antibiotic activity against *P. aeruginosa*.

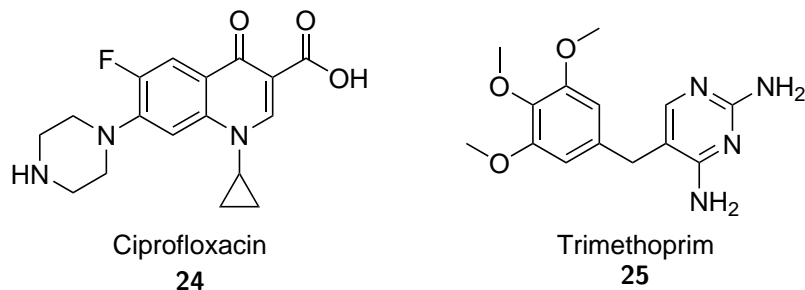


Figure 10: The antibiotics used in this section.



### 5.3.5 Antibiotic efflux

Ciprofloxacin **24** enters *P. aeruginosa* by diffusion,<sup>137</sup> but is pumped out by efflux pumps.<sup>138</sup> In the planktonic state several efflux pumps are known to pump out ciprofloxacin **24**, including MexAB–OprM, MexCD–OprJ, MexEF–OprN, MexXY–OprM, MexJK–OprM and MexVW–OprM.<sup>8</sup> However, in biofilms only MexEF–OprN has an effect.<sup>139</sup>

Trimethoprim **25** is mainly exported by the MexAB–OprM,<sup>140</sup> MexCD–OprJ<sup>141</sup> and MexEF–OprN<sup>142</sup> multidrug efflux systems<sup>8,143</sup> in the planktonic state. It is not known which pumps are used to export trimethoprim **25** from biofilms, but biofilms do show increased resistance to it.<sup>96</sup>

### 5.3.6 Conjugate efflux and antibiotic action

There are two synergistic mechanisms by which the conjugates could disrupt *P. aeruginosa* growth:

1. *P. aeruginosa* could develop resistance to an autoinducer-antibiotic conjugate by upregulation of the autoinducer’s export mechanism, but this would also lead to increased export of the native autoinducer, thus disrupting the quorum sensing system and hence biofilm formation and virulence.<sup>88,131,132</sup> For HSL conjugates this would mean upregulation of the MexAB–OprM pump, as this is the pump used for export of 3-oxo-C<sub>12</sub>-HSL **20**.<sup>8,131</sup> For PQS conjugates this would mean upregulation of vesicle formation.<sup>129</sup>
2. The autoinducer section could make the conjugate a poor substrate for the antibiotic section’s usual efflux mechanism, leading to accumulation of the conjugate within cells and hence increased antibacterial activity. For autoinducer-ciprofloxacin conjugates acting on planktonic *P. aeruginosa* this would mean the conjugate being a poor substrate of the various efflux pumps listed in the previous section. For autoinducer-ciprofloxacin conjugates acting on biofilms this would mean the conjugate being a poor substrate of MexEF–OprN (the sole exporter of ciprofloxacin **24** in biofilms<sup>139</sup> and not an exporter of HSLs **19** or **20**, or PQS **22**<sup>8</sup>). This mechanism could in principal work for trimethoprim **25** as well, but it is not known which pumps are active against this antibiotic in biofilms.

It is worth noting that for either of these mechanisms we are primarily interested in the autoinducer’s interaction with its import/export mechanism, rather than its receptor in the quorum sensing system. However, binding to receptors could help retention within the cell, so either way it is important that both the autoinducer and antibiotic sides of the conjugates closely resemble the unconjugated molecules. With this in mind, an initial library was designed using a copper(I)-catalysed azide-alkyne cycloaddition reaction<sup>144,145</sup> to join each combination of autoinducer and antibiotic together, using relatively long linkers in order to stop one side interfering with the binding of the other to its target protein.

### 5.3.7 Cleavable linkers

As part of the library, a set of cleavable HSL-Cip triazole conjugates was synthesised in collaboration with Professor Eddy Sotelo. These were based on the cleavable pyochelin-norfloxacin conjugates synthesised by Rivault *et al.*<sup>50</sup> (see Figure 11). It was envisaged that the linker would be stable under the extracellular assay conditions, but would be cleaved upon entry into the cell by intracellular esterases. It was hoped that the attached HSLs would improve retention of the conjugate in cells.

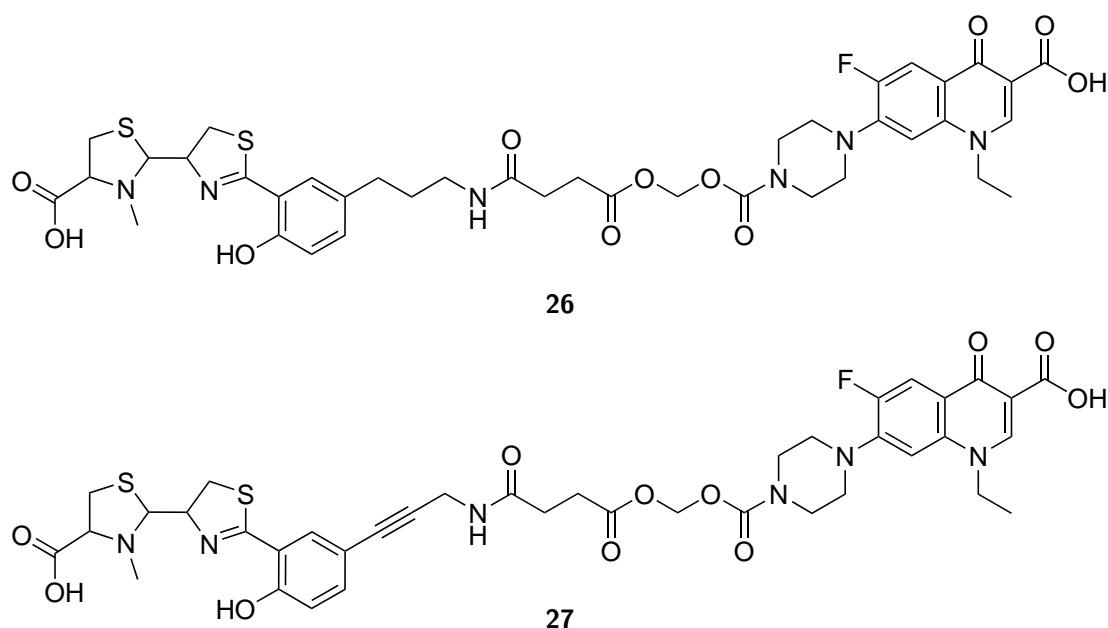


Figure 11: The cleavable pyochelin–norfloxacin conjugates synthesised by Rivault *et al.*<sup>50</sup>

The properties of similar linkers (see Figure 12, R = Me) were studied by Gogate *et al.*, who found that they were stable for more than 3 years under optimal conditions.<sup>146</sup> The hydrolysis of a secondary amine prodrug is dependent on ester hydrolysis rate, therefore the cleavage rate can be tuned by changing the R group between the ester and amide.<sup>147</sup> The *N*-(acetoxymethoxycarbonyl) (R = Me) linkers have been shown to be cleaved by esterases at an enhanced rate compared to buffer, and thus show promise in prodrugs.<sup>148</sup> It was therefore hoped that they will allow intracellular release of the ciprofloxacin **24** payload from the conjugates in this study. Both the *N*-(acetoxymethoxycarbonyl) (R = H) and *N*-(acetoxymethoxycarbonyl) (R = Me) were used, to investigate whether differences in cleavage rate could tune activity.

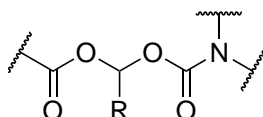


Figure 12: The cleavable linkers investigated in this study.

### 5.3.8 Homoserine lactone analogue-ciprofloxacin conjugates

Following on from the library of compounds based on *P. aeruginosa* autoinducers, a series of conjugates based on analogues of HSL were planned. This strategy was inspired by a paper<sup>61</sup> and patent<sup>149</sup> by Ganguly *et al.*, who synthesised and characterised a conjugate **154** of methyl ciprofloxacin **151** with homocysteine thiolactone (see Figure 13). Homocysteine thiolactone is an analogue of HSL with the ring oxygen replaced by sulfur, and has been used as the head group in several other known quorum sensing modulators.<sup>80,150–156</sup>

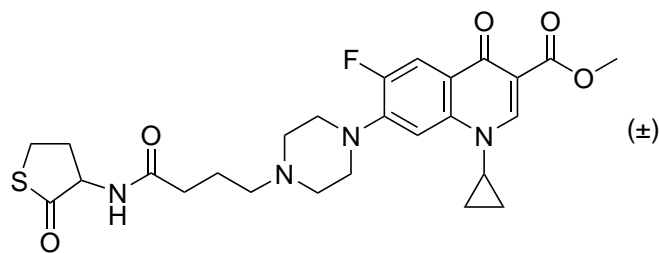


Figure 13: The HCTL-CipMe conjugate **154** studied by Ganguly *et al.*<sup>61, 149</sup>

As part of their characterisation of the HCTL-CipMe conjugate **154**, Ganguly *et al.* found the minimum inhibitory concentration (MIC) of the conjugate in *P. aeruginosa* under standard planktonic conditions. The MIC was found to be ten times higher for the conjugate vs. ciprofloxacin **24** (50 vs. 5  $\mu\text{m}$ ), indicating that the conjugate was less effective than ciprofloxacin **24** under planktonic conditions.

Ganguly *et al.* then investigated the effect of the conjugate on biofilms. The conjugate **154** and ciprofloxacin **24** were first added to dilute *P. aeruginosa* liquid culture at 25  $\mu\text{m}$ . As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin **24**, but did grow in the presence of the conjugate **154**. They then incubated cultures for 24 h, to allow biofilms to grow, before adding the compounds. In contrast, they found that the conjugate **154** disrupted the biofilm more effectively than ciprofloxacin **24**. When the biofilm was grown for 48 or 72 hours the conjugate had similarly disruptive effects, whereas ciprofloxacin **24** ‘did not show any significant antibacterial activity’.

These results are exciting as they hint that an autoinducer conjugate might be able to combat an established *P. aeruginosa* infection more effectively than the unmodified antibiotic. Ganguly *et al.* suggest that their conjugate **154** is more effective than ciprofloxacin **24** in penetrating biofilms, and/or better at avoiding being pumped out by multidrug efflux pumps. They posit that this could be due to the thiolactone head, as they also showed that unconjugated C<sub>4</sub>-HCTL **28** (see Figure 14) has ‘either enhanced uptake or functional activity’ when compared with C<sub>4</sub>-HSL **19**.

It is possible that the conjugate **154** has higher activity against biofilms when compared with ciprofloxacin **24** because the conjugate **154** avoids being pumped out by multidrug efflux pumps, or selects for the survival of mutants with upregulated efflux pumps, and hence disrupts quorum sensing systems (see 5.3.6).

While one might expect the conjugate **154** to behave like C<sub>4</sub>-HSL **19**, and hence passively diffuse in and out of cells, it is possible that its transport more closely resembles that of 3-oxo-C<sub>12</sub>-HSL **20**. 3-oxo-C<sub>12</sub>-HSL **20**’s accumulation in membranes and interaction with efflux pumps is thought to be based primarily on tail chain length (see 5.3.3), and the ciprofloxacin half of the conjugate **154** could be seen as a long tail, especially as the carboxylic acid is methylated and hence less polar.

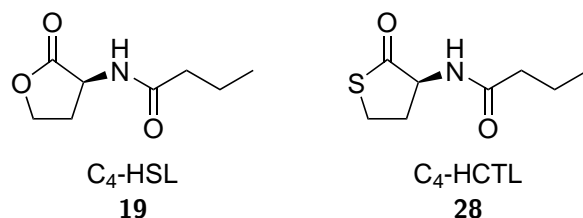


Figure 14: C<sub>4</sub>-HSL **19** and C<sub>4</sub>-HCTL **28**. Note that Ganguly *et al.* tested the *S* enantiomer of C<sub>4</sub>-HCTL **28**, but used a racemic mixture in their HCTL-CipMe conjugate.

While the results found by Ganguly *et al.* show promise, they only test one conjugate, and do not include controls to show that the HCTL group specifically is necessary for the enhanced effect. It was therefore decided

to build on this work by synthesising a series of ciprofloxacin conjugates with head groups taken from known quorum sensing modulators,<sup>128, 157</sup> a selection of which are described in Table 2.

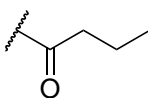
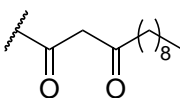
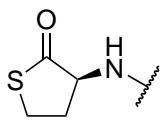
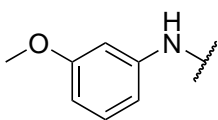
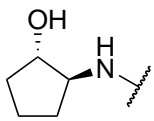
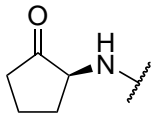
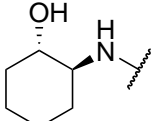
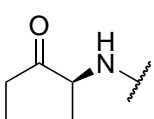
Head group		
	Partial agonist and antagonist against LasR. <sup>154</sup> Shown to increase biofilm formation in <i>P. aeruginosa</i> . <sup>61</sup>	Strong agonist against LasR, with comparable activity to the native ligand. <sup>151, 152, 154, 158</sup>
	Partial agonist against LasR. <sup>157</sup>	Strong antagonist against LasR. <sup>157</sup>
	Poor agonist and antagonist against RhlR. <sup>159, 160</sup>	Strong antagonist against LasR. <sup>159</sup>
	Strong agonist against RhlR. <sup>159</sup> <i>SS</i> enantiomer is more potent. <sup>160</sup>	Partial agonist against LasR. <sup>159</sup>
	Strong agonist against RhlR. <sup>159</sup> <i>SS</i> enantiomer is more potent, with comparable activity to the native ligand. <sup>160</sup>	Strong agonist against LasR. <sup>152, 159</sup> <i>SS</i> enantiomer is more potent, with comparable activity to the native ligand. <sup>160</sup>
	Strong agonist against RhlR. <sup>159</sup> <i>SS</i> enantiomer is more potent. <sup>160</sup>	Partial antagonist against LasR. <sup>159</sup> Shown to reduce biofilm formation in <i>P. aeruginosa</i> . <sup>159</sup>

Table 2: Activities of quorum sensing modulators containing the head groups used in this study.

## 6 Project aims and summary

The aim of this project is to produce and test a library of autoinducer-antibiotic conjugates with the goal of producing conjugates with greater potency than the parent antibiotics. The work is divided into two main sections. Section 7 focuses on conjugates of three *P. aeruginosa* autoinducers (see Figure 8) with ciprofloxacin **24** and trimethoprim **25** (see Figure 10) joined using a copper(I)-catalyzed azide-alkyne cycloaddition. Section 8 focuses on conjugates of HSL analogues with ciprofloxacin **24** (see 5.3.8) joined either using a copper(I)-catalyzed azide-alkyne cycloaddition or an  $S_N2$  reaction or peptide coupling.

## 7 Results and discussion: autoinducer-antibiotic conjugates

### 7.1 Overview

The first part of this project was focused on producing a library of autoinducer-antibiotic conjugates. *P. aeruginosa* autoinducers were used, in particular C<sub>4</sub>-HSL **19**, HHQ **21** and PQS **22** (see Figure 8). Azido derivatives of these compounds were coupled to alkynyl derivatives of antibiotics, specifically ciprofloxacin **24** and trimethoprim **25** (see Figure 10), using a copper(I)-catalysed azide-alkyne cycloaddition.<sup>144,145</sup> The compounds were then tested for antibiotic and anti-biofilm activity against *P. aeruginosa*. The decisions on where to attach the azide or alkyne handles to the chosen molecules are discussed below.

#### 7.1.1 Azido autoinducer derivatives

The structure-activity relationships in HHQ **21** and PQS **22** have been previously studied,<sup>161–163</sup> and it was shown various substitutions on the benzene ring could be made without significantly decreasing activity. The 6-azido derivatives (see Figure 15) were chosen for this study as routes to them have previously been found.<sup>164</sup>

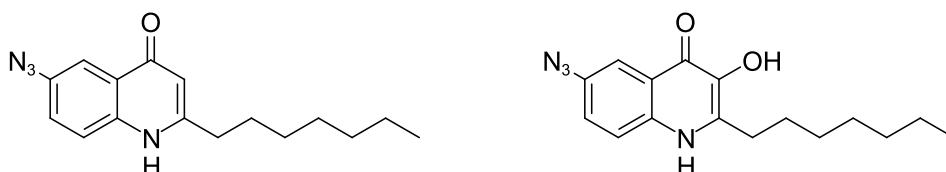


Figure 15: The azido derivatives of HHQ **21** and PQS **22**: **38** and **49**.

Alteration of the lactone group of HSL derivatives is known to significantly decrease activity, especially where the number of H-bond donors or acceptors is altered.<sup>128</sup> Hence, the azide group was included on the tail.<sup>165</sup> Acyl tail length is known to play an important role in affinity,<sup>128</sup> so three derivatives of C<sub>4</sub>-HSL **19** were synthesised: N<sub>3</sub>-C<sub>2</sub>-HSL **55**, N<sub>3</sub>-C<sub>4</sub>-HSL **58** and N<sub>3</sub>-C<sub>6</sub>-HSL **61** (see Figure 16).

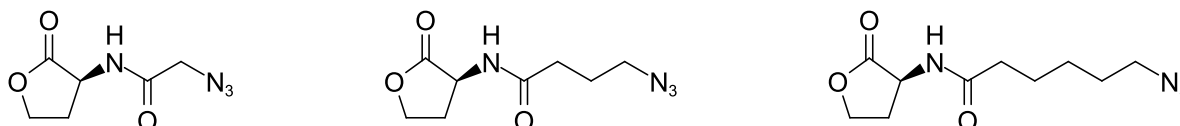


Figure 16: The azido derivatives of C<sub>4</sub>-HSL **19**: **55**, **58** and **61**.

#### 7.1.2 Alkynyl antibiotic derivatives

The structure-activity relationships for ciprofloxacin **24** have been investigated<sup>166</sup> and modifications at the cyclopropane and piperazine groups were found not to cause loss of activity. It was decided an alkyne tail would be added onto the free NH of the piperazine ring, as this position is more synthetically accessible. Alkynyl ciprofloxacin derivative **68** (see Figure 17) was synthesised in this study (see 7.3.1), and two cleavable alkynyl ciprofloxacin derivatives **90** and **91** were synthesised by Professor Eddy Sotelo and combined with the azido HSL derivatives described above to create cleavable conjugates (see 7.4.3).

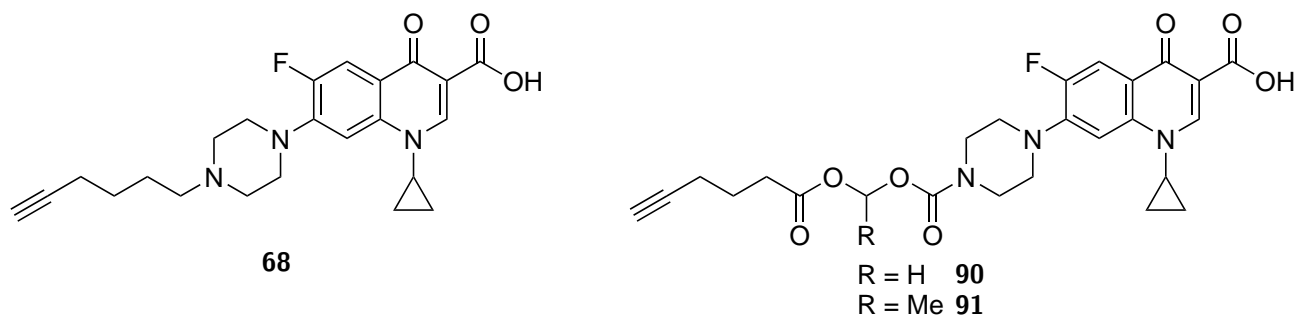


Figure 17: The alkyne ciprofloxacin derivatives **68**, **90** and **91**.

The choice to of alkyne tail attachment point on trimethoprim **25** (see Figure 18) is based on the use of that same point in a fluorogenic trimethoprim tag synthesised by Jing *et al.*<sup>167</sup>

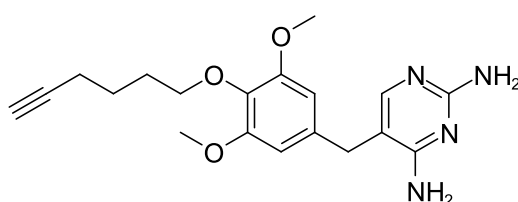
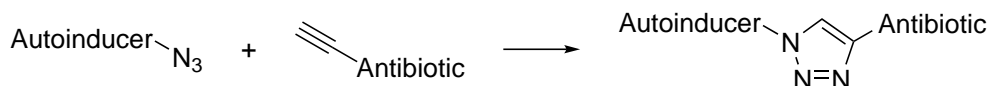


Figure 18: The alkyne trimethoprim derivative **71**.

### 7.1.3 Synthesis of the conjugates

A copper(I)-catalysed azide-alkyne cycloaddition<sup>144, 145</sup> was used to join each combination of autoinducer and antibiotic together (see Scheme 1).



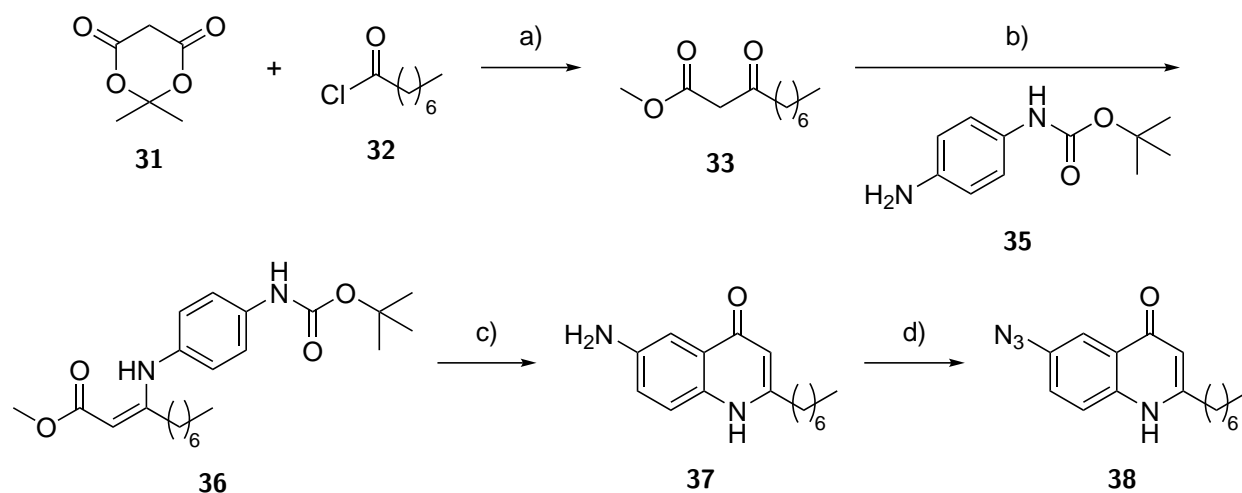
Scheme 1: The construction of the triazole-linked autoinducer-antibiotic conjugate library using a copper(I)-catalysed azide-alkyne cycloaddition.

## 7.2 Synthesis of the azido autoinducer derivatives

### 7.2.1 Synthesis of 6-N<sub>3</sub>-HHQ **38**

The synthesis of 6-N<sub>3</sub>-HHQ **38** is shown in Scheme 2 and follows a route devised by Baker.<sup>164</sup> Octanoyl chloride **32** was converted to  $\beta$ -ketoester **33** via a Meldrum's acid adduct.<sup>168, 169</sup> The  $\beta$ -ketoester **33** was condensed with *N*-Boc-*para*-phenylenediamine **35** to form enamine **36**. The disappointing yield of this step was in part due to the reaction proceeding to an equilibrium state rather than to completion, and hence not all of the starting material being consumed; starting materials can be recycled to improve the yield. Alternatively, Baker later found a higher-yielding reaction using a ZrCl<sub>4</sub> catalyst.<sup>164</sup>

The enamine **36** was cyclised with polyphosphoric acid to form amino-HHQ **37** in good yield. The amine group of amino-HHQ **37** was converted to a diazo group by reaction with NaNO<sub>2</sub> and HCl, followed by displacement with NaN<sub>3</sub> to form the final azido-HHQ product **38**.<sup>170</sup>



Scheme 2: The synthesis of **38**. a) i) Pyridine,  $\text{CH}_2\text{Cl}_2$ , 0 °C. ii) MeOH, reflux, 66% over two steps. b) MeOH, reflux, 19%. c) Polyphosphoric acid, 120 °C, 72%. d) i)  $\text{NaNO}_2$ , HCl, water, 0 °C. ii)  $\text{NaN}_3$ , water, r.t., 41%.

### 7.2.2 Synthesis of 6- $\text{N}_3$ -PQS **49**

The synthesis of 6- $\text{N}_3$ -PQS **49** is shown in Scheme 3, and also follows a route devised by Baker.<sup>164</sup> The Weinreb amide **43**<sup>89</sup> was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide **40** to form 1-chlorononan-2-one **44** following a procedure described by Hodgkinson *et al.*<sup>171</sup>

The synthesis of PQS **22** described by Hodgkinson *et al.*<sup>171</sup> used a microwave reaction of 1-chlorononan-2-one **44** with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid **45** in the place of anthranilic acid in this microwave reaction, so that the nitro group could then be converted to an azide group via an amine. However, the microwave-catalysed reaction failed when 5-nitroanthranilic acid **45** was used.<sup>164</sup> Therefore, a two step process was employed instead.

5-Nitroanthranilic acid **45** was heated with  $\text{K}_2\text{CO}_3$  to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **44** to form the ester **46** by  $\text{S}_\text{N}2$  displacement of the chlorine atom in a procedure adapted from Hlaváč *et al.*<sup>172</sup> Cyclisation with polyphosphoric acid produced nitro-PQS **47** cleanly.<sup>172, 173</sup>

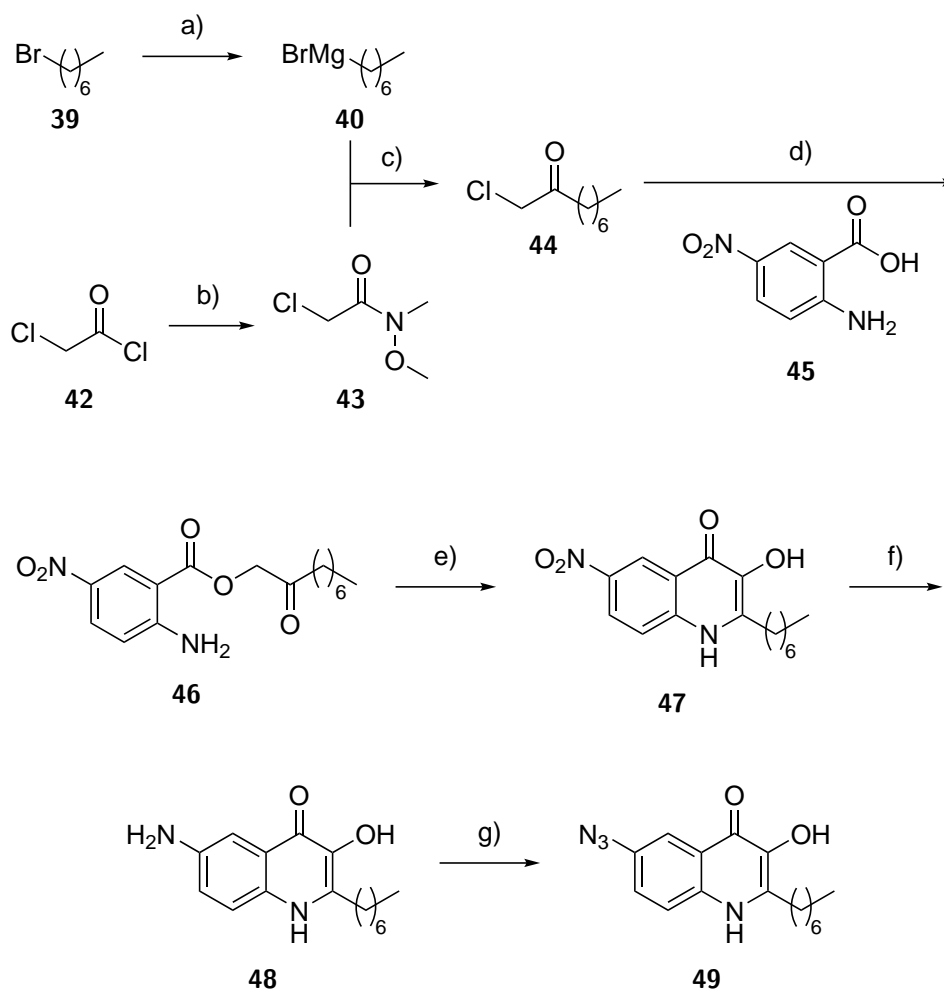
Conditions for the reduction of the nitro group were then compared (see Table 3). Baker initially used Zn and HCl, however this gave a yield over 100% suggesting coordination of Zn to the amino-PQS **48**<sup>164</sup> (this product was taken through and purified after the next step). She also attempted reduction with Pd/C and  $\text{H}_2$  or ammonium formate, but no reaction was observed.

Further conditions were tested in *this* work in order to obtain a clean sample of amino-PQS **48**. An initial test of reduction with  $\text{SnCl}_2$  produced no detectable product by LCMS. Catalytic hydrogenation using harsher conditions was then attempted, and it was determined that increasing the pressure to 3 atm using a Paar hydrogenator causes full conversion in 4 h using Pd/C and  $\text{H}_2$ . Good yields (80%) were also achieved using  $\text{PtO}_2$  as a catalyst, with the advantage that the reaction proceeds more quickly, and at atmospheric pressure and temperature.<sup>174</sup>

Finally, amino-PQS **48** was converted to azido-PQS **49** by reaction with  $\text{NaNO}_2$  and HCl to form diazo-PQS, followed by displacement of the diazo group using  $\text{NaN}_3$  to give the azido-PQS **49**.<sup>170</sup> The yield of this reaction was rather disappointing (28%), and is probably due to loss of product in the supernatant following precipitation.<sup>164</sup>

Conditions	Outcome
H <sub>2</sub> , Pd/C, 1 atm, r.t., 18 h	No reaction
NH <sub>4</sub> HCO <sub>2</sub> , Pd/C, 1 atm, r.t., 18 h	No reaction
Zn, HCl (aq), r.t., 5 min h	Product <b>48</b> + Zn, assumed quantitative yield
SnCl <sub>2</sub> · 2 H <sub>2</sub> O, MeOH, r.t., 18 h	No reaction
H <sub>2</sub> , Pd/C, MeOH, 3 atm, r.t., 4 h.	Product <b>48</b> , >99% yield
H <sub>2</sub> , PtO <sub>2</sub> , MeOH, 1 atm, r.t., 45 min	Product <b>48</b> , 80% yield

Table 3: Conditions attempted for the synthesis of **48**. Rows 1-3 were carried out by Baker,<sup>164</sup> rows 4-6 were carried out as part of this study.

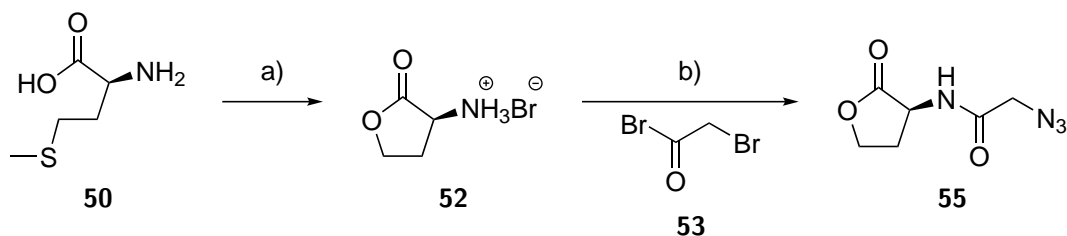


Scheme 3: The synthesis of **49**. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) *N,O*-dimethylhydroxylamine hydrochloride, K<sub>2</sub>CO<sub>3</sub>, toluene, water, - 5 °C to r.t., 30 min, 71%. c) THF, 0 °C to r.t., 15 h, 96%. d) **45**, K<sub>2</sub>CO<sub>3</sub>, DMF, 90 °C, 1 h, then **44**, r.t., 18 h, >99%. e) Polyphosphoric acid, 90 °C, 5.5 h, 40%. f) H<sub>2</sub>, PtO<sub>2</sub>, MeOH, 1 atm, r.t., 45 min, 80%. g) i) NaNO<sub>2</sub>, HCl, water, 0 °C, 50 min. ii) NaN<sub>3</sub>, water, r.t., 4 h, 28% over two steps.



### 7.2.3 Synthesis of the azido C<sub>4</sub>-HSL derivatives **55**, **58** and **61**

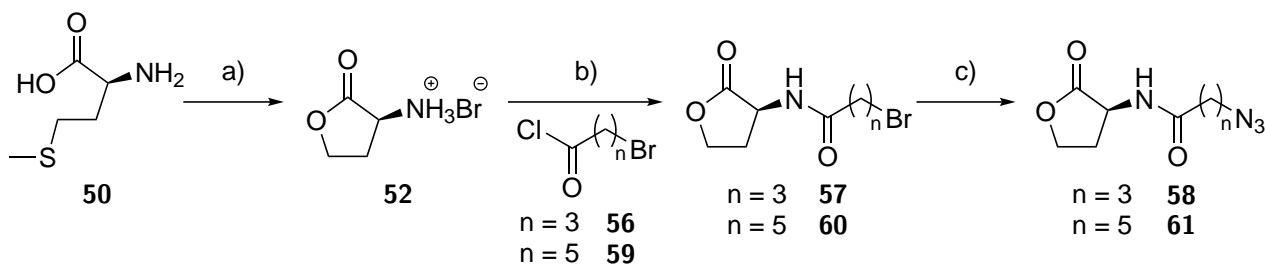
N<sub>3</sub>-C<sub>2</sub>-HSL **55** (the azido derivative of C<sub>4</sub>-HSL with a C<sub>2</sub> chain, see Scheme 4) has previously been prepared by Stacy *et al.*<sup>165</sup> Their synthesis was followed, starting with the cyclisation of L-methionine **50** using bromoacetic acid to form the HSL HBr salt **52**. The disappointing yield can be attributed to difficulties in precipitating the final product. The HSL HBr salt **52** was then converted by a biphasic one-pot process to N<sub>3</sub>-C<sub>2</sub>-HSL **55** using bromoacetyl bromide **53** and NaN<sub>3</sub>.



Scheme 4: The synthesis of **55**. a) Bromoacetic acid, *i*-PrOH:water:AcOH (5:5:2), r.t., 18 h, 41%. b) NaN<sub>3</sub>, NaHCO<sub>3</sub>, water/CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, 41%.

It was hoped that this procedure could also be used to produce the C<sub>4</sub> and C<sub>6</sub> derivatives, however, attempts to convert HSL **50** to N<sub>3</sub>-C<sub>4</sub>-HSL **58** using 4-bromobutyryl chloride **56** produced a complex mixture of products. This is likely to be because the S<sub>N</sub>2 reaction in which the azide anion displaces bromine is slower for the C<sub>4</sub> derivative as the bromine atom being displaced is no longer adjacent to a carbonyl group. In addition, the longer chain length allows intramolecular cyclisation of the bromide with the secondary amide. The conversion was therefore carried out as a two-step process, where a bromoacyl chain was initially installed, followed by the S<sub>N</sub>2 reaction with NaN<sub>3</sub> (see Scheme 5).

Reaction of the HSL HBr salt **52** with 4-bromobutyryl chloride **56** or 6-bromohexanoyl chloride **59** produced Br-C<sub>4</sub>-HSL **57** or Br-C<sub>6</sub>-HSL **60** respectively, in good yields. Heating with NaN<sub>3</sub> in DMF converted Br-C<sub>6</sub>-HSL **60** to N<sub>3</sub>-C<sub>6</sub>-HSL **61**. Similar conditions were used by Dr Bin Yu, a visiting PhD student in the Spring group, to convert the bromo-C<sub>4</sub> derivative **57** to the azido-C<sub>4</sub> derivative **58**, and this compound was kindly donated to complete the set. Yields for the S<sub>N</sub>2 reaction could probably be improved by decreasing the temperature (see Scheme 22, for example).



Scheme 5: The synthesis of **58** and **61**. a) Bromoacetic acid, *i*-PrOH:water:AcOH (5:5:2), r.t., 18 h, 41%. b) NaHCO<sub>3</sub>, water/CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, **57**: 80%, **60**: 66%. c) NaN<sub>3</sub>, DMF, 100 °C, 5 h, **61**: 27% (donated by Dr Bin Yu), **61**: 56%.

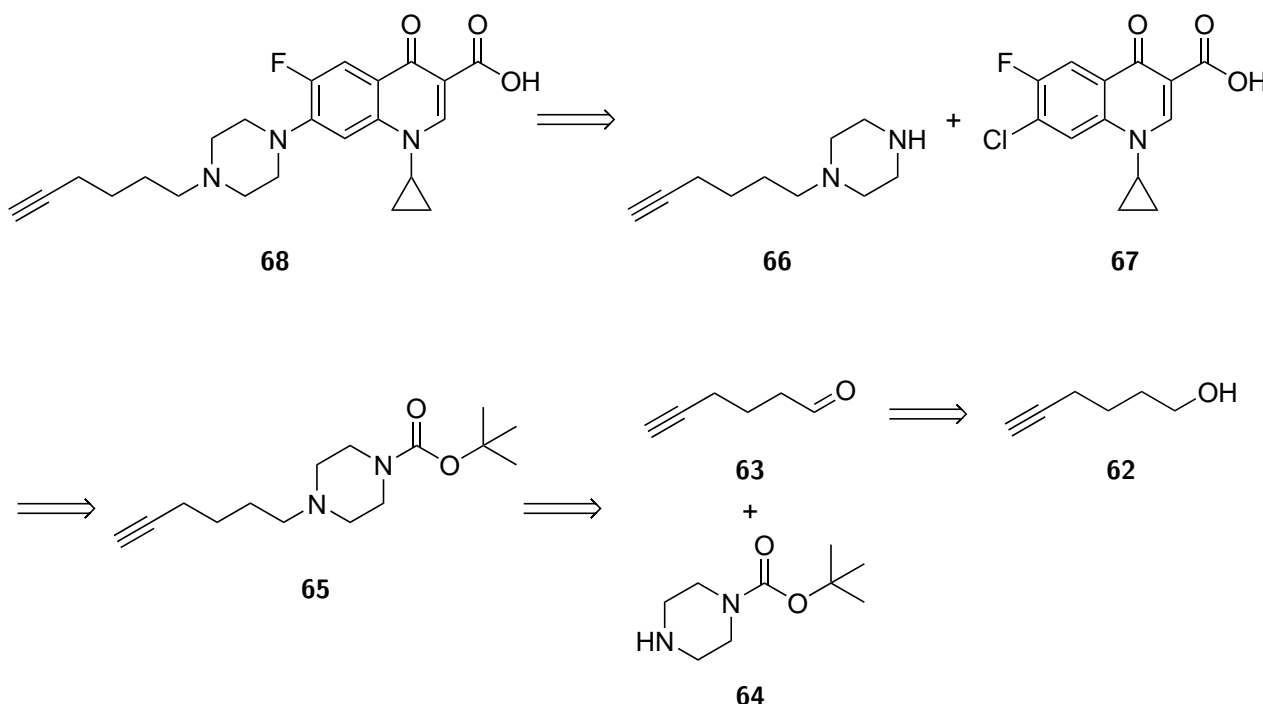
## 7.3 Synthesis of the alkynyl antibiotic derivatives

### 7.3.1 Synthesis of the alkynyl ciprofloxacin derivative **68**

The retrosynthesis of ciprofloxacin derivative **68** is shown in Scheme 6. The disconnection to an alkynyl piperazine **66** and a commercially available ciprofloxacin precursor **67** was chosen based on a study by Renau *et al.*, who found this route to be “...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide.”<sup>166, 175</sup>

It was envisaged that the alkynyl piperazine **68** could be prepared from mono-Boc-protected piperazine **64** and hex-5-ynal **63** using conditions similar to those used by Renau *et al.*<sup>166</sup>

Unlike the aldehydes and ketones used by Renau *et al.*,<sup>166</sup> hex-5-ynal **63** is not commercially available and so it was hoped that this could be prepared by oxidation of hex-5-ynol **62**.



Scheme 6: The retrosynthesis of **68**.

The synthesis of ciprofloxacin derivative **68** is shown in Scheme 7. Hex-5-ynal **63** was prepared by pyridinium chlorochromate oxidation of hex-5-ynol **62** in good yield according to the procedure described by Kocsis *et al.*<sup>176</sup>

Renau *et al.*<sup>166</sup> used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal **63** and 1-Boc-piperazine **64**. However, it was decided to attempt this transformation using the less toxic sodium triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*<sup>177</sup> This reaction yielded compound **65** in excellent yield, which was deprotected using TFA using the procedure described by Renau *et al.*<sup>166</sup> to give the alkynyl piperazine **66** quantitatively.

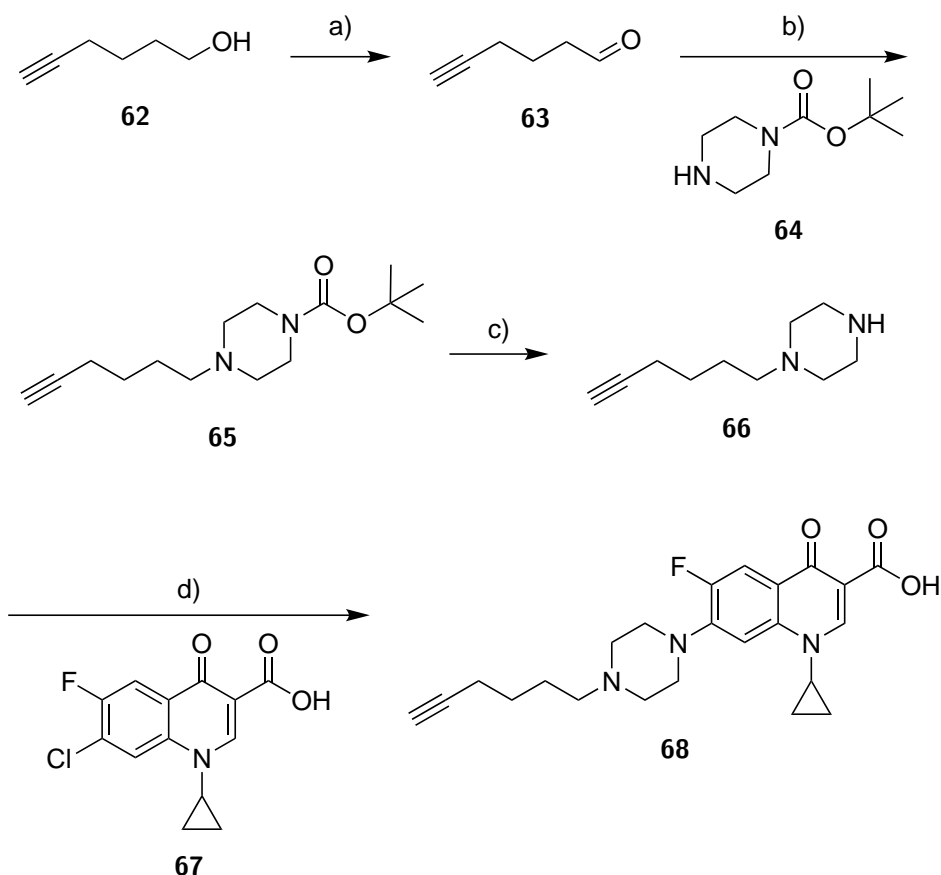
The alkynyl piperazine **66** was refluxed in acetonitrile with the ciprofloxacin precursor **67** according to the procedure described by Renau *et al.*,<sup>166</sup> however the reaction did not proceed. Addition of 2 eq. of TEA did not lead to reaction, however it was found that refluxing in neat TEA led to conversion to the final ciprofloxacin derivative **68**.

With a small sample of the final product in hand, less harsh conditions were sought for a larger-scale version of the final reaction. Microwave irradiation at 115 °C was used, following a procedure by Reddy *et al.*<sup>178</sup> DMSO and NMP were tested as solvents, with or without the addition of TEA. The reactions were monitored using

LCMS, and NMP without TEA was found to give the highest conversion.

Work-up of this reaction proved challenging, with an unknown dark brown viscous liquid being formed which was difficult to separate from the white solid product. A pure sample was obtained by recrystallisation from EtOAc, but the yield was poor (12%). The reaction was observed to stall after a certain point, while still having some of the ciprofloxacin precursor **67** present. The alkynyl piperazine **66** was not observed by TLC despite having been added in two-fold excess, suggesting that it degraded to a by-product before having chance to react.

Further attempts to refine this reaction might involve lower temperatures, higher ratios of the alkynyl piperazine **66** or improvement of the purification, e.g. by finding better precipitation conditions or by using reverse-phase chromatography. A Buchwald-Hartwig coupling or Ullmann reaction could also be attempted, but, as seen later, coordination of ciprofloxacin **24** to Cu can hinder catalysis.

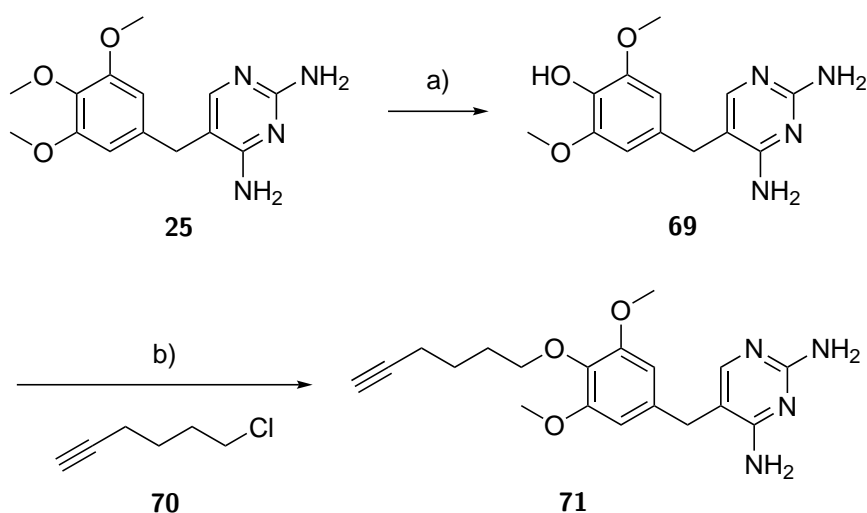


Scheme 7: The synthesis of **68**. a) Pyridinium chlorochromate, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 5 h, 72%. b) NaBH(AcO)<sub>3</sub>, 1,2-dichloroethane, r.t., 10.5 h, 99%. c) TFA, r.t., 1 h, >99%. d) NMP, microwave, 115 °C 24 h, 12%.

### 7.3.2 Synthesis of the alkynyl trimethoprim derivative **71**

The synthesis of trimethoprim derivative **71** is shown in Scheme 8. Trimethoprim **25** was selectively deprotected using HBr (aq.) using a procedure described by Jing *et al.*<sup>167</sup> to form **69**. A slightly longer reaction time (40 min vs 20 min) probably led to the yield being somewhat lower than that obtained by Jing *et al.*. The main impurity was asymmetrically di-demethylated trimethoprim, which could be identified by the presence of two aryl peaks at 6.41 (d, J=2.0 Hz, 1 H) and 6.34 (d, J=2.0 Hz, 1 H) and a corresponding methyl peak at 3.82 (s, 3 H) in the crude NMR.

The alkynyl trimethoprim derivative **71** was synthesised from the demethylated trimethoprim **69** and 6-chloro-1-hexyne **70** using a Cs<sub>2</sub>CO<sub>3</sub>-catalysed S<sub>N</sub>2 reaction similar to that used by Jing *et al.*<sup>167</sup>



Scheme 8: The synthesis of **71**. a) HBr (aq.), 100 °C, 40 min, 43%. b) Cs<sub>2</sub>CO<sub>3</sub>, DMF, 70 °C, 7 h, 25%.

## 7.4 Synthesis of the triazole-linked autoinducer-antibiotic conjugates

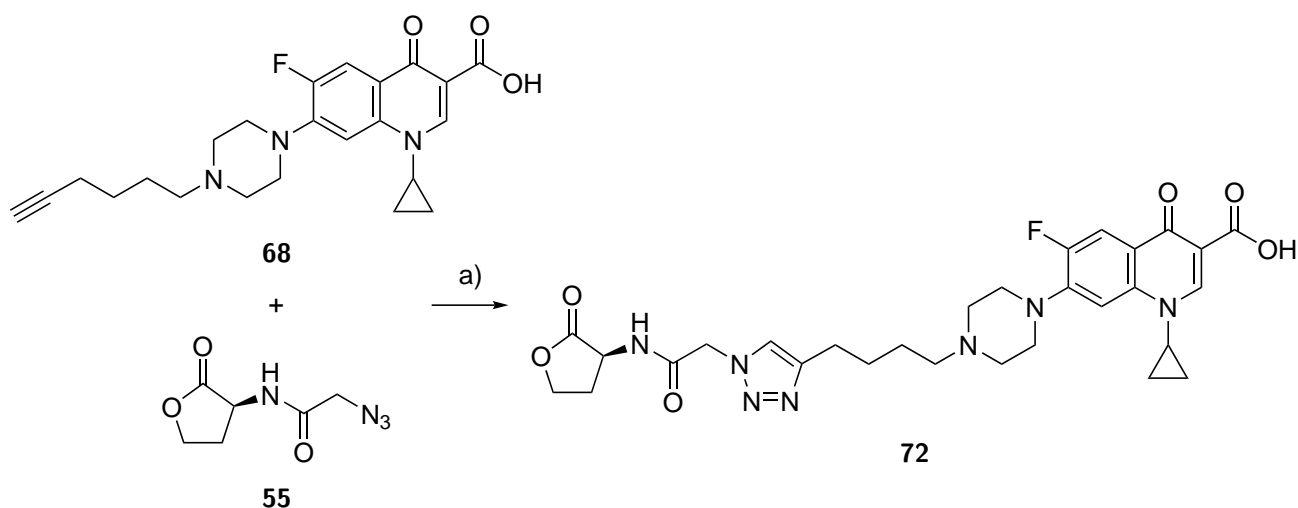
### 7.4.1 Optimisation of the click reaction

Test reactions using N<sub>3</sub>-C<sub>2</sub>-HSL **55** and the alkynyl ciprofloxacin derivative **68** were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see Table 4 and Scheme 9). Stirring at r.t. had no effect even with an extended reaction time. Heating to 50 °C did lead to slow formation of the product, but a mixture of the 1,4 **72** and 1,5 **73** isomers was observed in an approximately 4:1 ratio by LCMS (see Figure 20). It is possible that the Cu(I) catalyst was not involved in this reaction because it had been oxidised, and hence the mixture of products was formed by an uncatalysed cycloaddition. Such reactions are known to produce a mixture of products.<sup>145</sup>

Use of the ligand tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) **74** (see Figure 19) led to some conversion at room temperature (seen by LCMS), however the reaction stopped before completion, probably due to oxidation of the Cu(I) catalytic species. When degassed solvent and an argon atmosphere were used the reaction proceeded to completion at room temperature in around 3 h.

Conditions	Outcome
CuSO <sub>4</sub> · 5 H <sub>2</sub> O, sodium ascorbate, water, <i>t</i> -BuOH, air, r.t., 7 d.	No reaction
CuSO <sub>4</sub> · 5 H <sub>2</sub> O, sodium ascorbate, water, <i>t</i> -BuOH, air, 50 °C, 5 d.	1,3-Triazole product <b>72</b> and 1,5 triazole impurity <b>73</b> 4:1
CuSO <sub>4</sub> · 5 H <sub>2</sub> O, sodium ascorbate, THPTA <b>74</b> , water, <i>t</i> -BuOH, air, r.t., 3 h.	1,3-Triazole product <b>72</b> and starting materials <b>55</b> and <b>68</b> 5:4:4
CuSO <sub>4</sub> · 5 H <sub>2</sub> O, sodium ascorbate, THPTA <b>74</b> , water, <i>t</i> -BuOH, Ar, r.t., 3 h.	1,3-Triazole product <b>72</b> , 30% yield

Table 4: Conditions attempted for the synthesis of **72** (see Scheme 9).



Scheme 9: Synthesis of **72**. For conditions see Table 4.

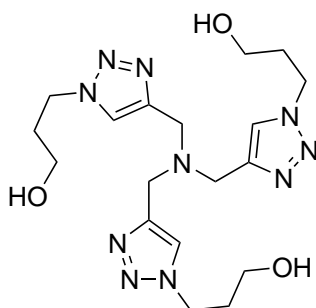


Figure 19: Tris[(1-benzyl- *1H*-1,2,3-triazol-4-yl)methyl]amine (THPTA) **74** .

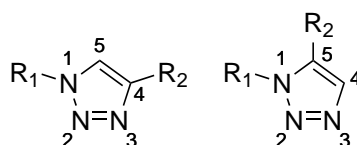


Figure 20: 1,4 (left) and 1,5 (right) triazoles.

#### 7.4.2 Synthesis of the autoinducer-ciprofloxacin and autoinducer-trimethoprim triazole conjugates

Once conditions had been found for the click reaction, the synthesis of other conjugates was attempted. Two additional azides were kindly donated by members of the Spring group: the azido derivative of 3-oxo-C<sub>12</sub>-HSL **75** was synthesised by Ryan Howard, a master's student under my supervision<sup>179</sup> and the tail azide derivative of PQS **76** was synthesised by Dr Ysobel Baker<sup>164</sup> (see Figure 21).

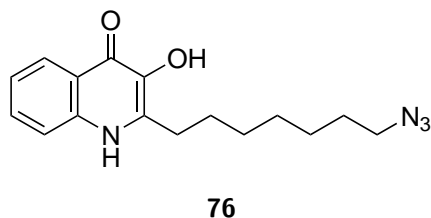
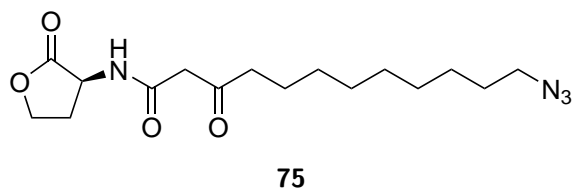
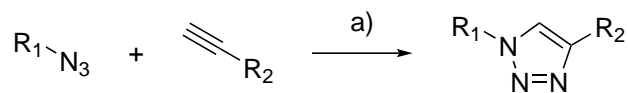


Figure 21: Further azido autoinducer derivatives synthesised by Howard<sup>179</sup> **75** and Baker<sup>164</sup> **76**.

Synthesis of the conjugates proved more difficult than expected, for several reasons. Firstly some compounds did not dissolve in the reaction solvent (50% water/*t*-BuOH) requiring addition of co-solvents such as CH<sub>2</sub>Cl<sub>2</sub>. Secondly, some compounds were unstable: HSL derivatives hydrolysed upon attempted preparative HPLC purification and the 3-oxo-C<sub>12</sub>-HSL conjugates degraded during the reaction. Finally, the reaction was highly air-sensitive which led to stalling. The most reliable procedure was determined over the course of several reactions, and is shown in 9.25.

Despite the unforeseen difficulties in synthesis of the conjugates enough material was successfully prepared for biological testing. The results of the reactions are shown in Table 5, Table 6, Table 7 and Table 8. It was intended that the failed reactions would be repeated, but as preliminary biological testing (see 7.5) proved unpromising it was decided that attention should be focused elsewhere.



Scheme 10: General scheme for the click reaction, where R<sub>1</sub>-N<sub>3</sub> is an azido autoinducer derivative and R<sub>2</sub>-≡ is an alkynyl antibiotic derivative a) CuSO<sub>4</sub>, sodium ascorbate, THPTA, water, *t*-BuOH.

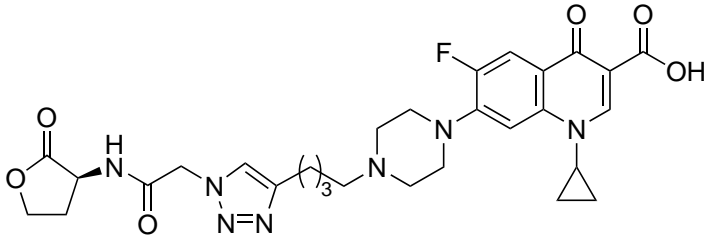
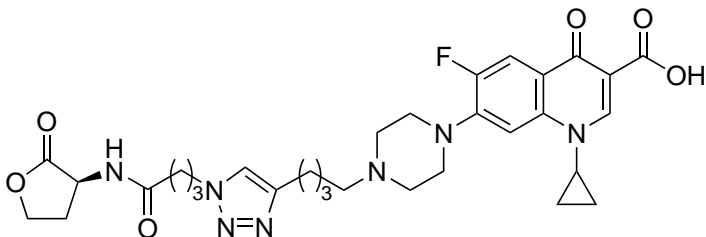
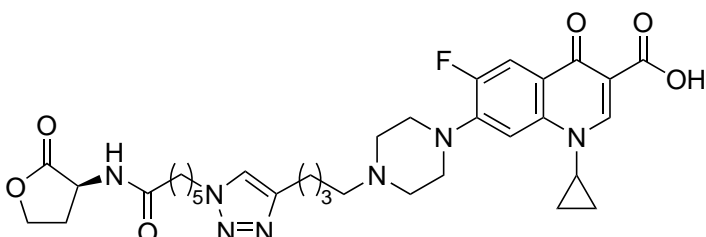
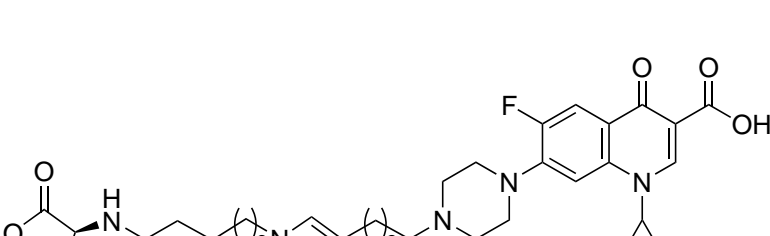
Starting materials	Product	Outcome	Yield
55 and 68	 <p style="text-align: center;"><b>72</b></p>	<p>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).</p>	30%
58 and 68	 <p style="text-align: center;"><b>77</b></p>	<p>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).</p>	47%
61 and 68	 <p style="text-align: center;"><b>78</b></p>	<p>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).</p>	38%
75 and 68	 <p style="text-align: center;"><b>79</b></p>	<p>✗ Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO<sub>2</sub>, 20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).</p>	

Table 5: Click reactions attempted.

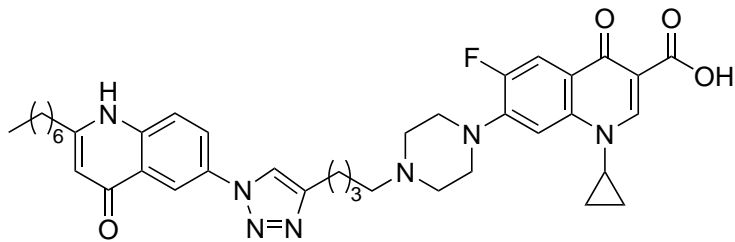
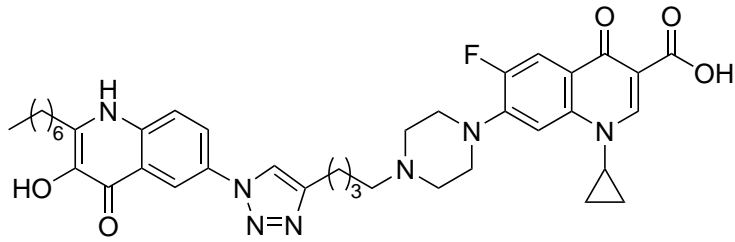
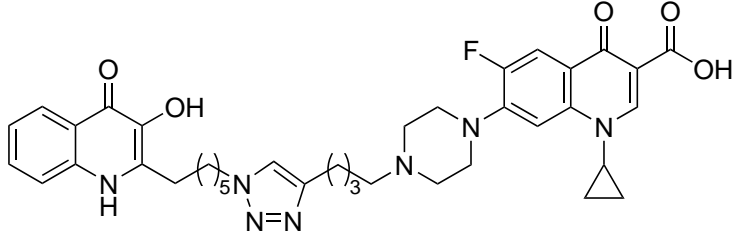
Starting materials	Product	Outcome	Yield
<b>38 and 68</b>	 <p style="text-align: center;"><b>80</b></p>	<p>✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.</p>	27%
<b>49 and 68</b>	 <p style="text-align: center;"><b>81</b></p>	<p>✗ Reaction did not go to completion (approx. 6:1 starting material <b>68</b> to product <b>81</b> seen by LCMS). Attempted purification by prep. HPLC but unsuccessful.</p>	
<b>76 and 68</b>	 <p style="text-align: center;"><b>82</b></p>	<p>✗ No reaction seen by LCMS.</p>	

Table 6: Click reactions attempted.



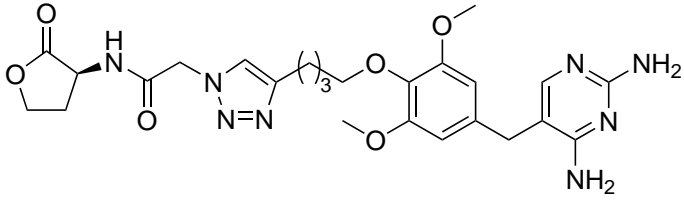
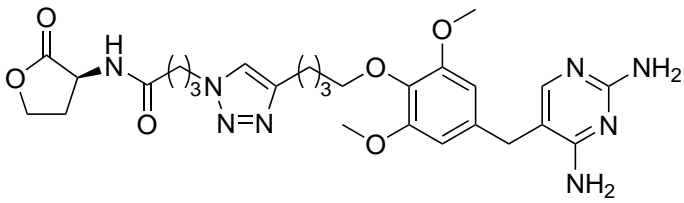
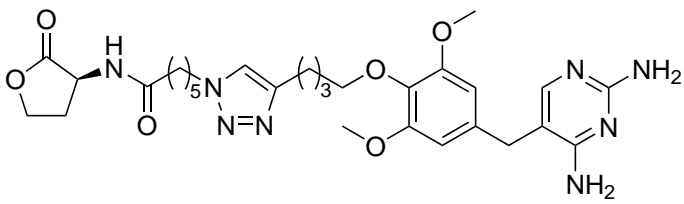
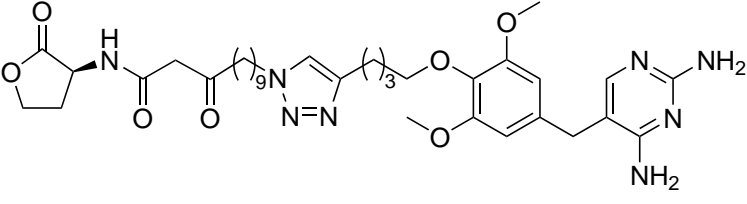
Starting materials	Product	Outcome	Yield
55 and 71	 <p style="text-align: center;"><b>83</b></p>	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on prep. HPLC column.	
58 and 71	 <p style="text-align: center;"><b>84</b></p>	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO <sub>2</sub> , 20% MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	17%
61 and 71	 <p style="text-align: center;"><b>85</b></p>	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO <sub>2</sub> , 20% MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	27%
75 and 71	 <p style="text-align: center;"><b>86</b></p>	✗ Degraded during reaction.	

Table 7: Click reactions attempted.

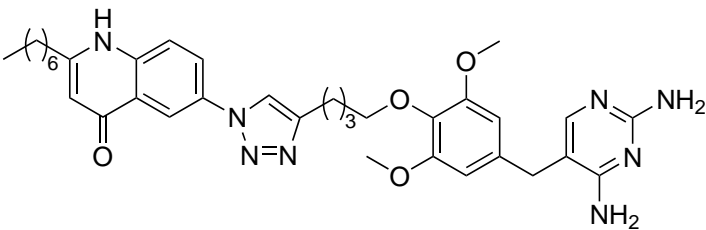
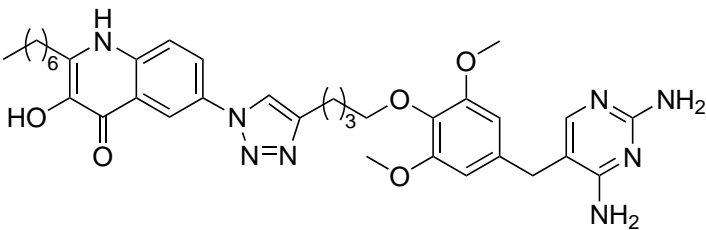
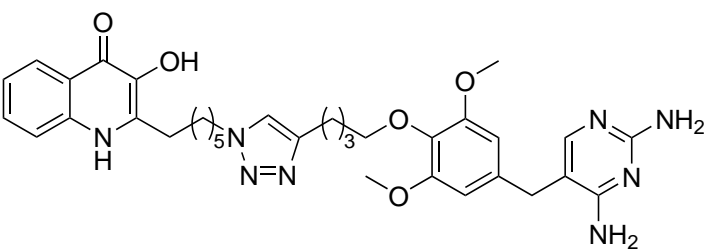
Starting materials	Product	Outcome	Yield
38 and 71	 87	<p>✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.</p>	41%
49 and 71	 88	<p>✗ Reaction did not go to completion (approx. 10:1 starting material 71 to product 88 seen by LCMS). Attempted purification by prep. HPLC but unsuccessful.</p>	
76 and 71	 89	<p>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO<sub>2</sub>, 20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).</p>	18%

Table 8: Click reactions attempted.

#### 7.4.3 Synthesis of the homoserine lactone-ciprofloxacin triazole conjugates with cleavable linkers

In addition to the conjugates shown in the previous section, a further collection was synthesised in collaboration with Professor Eddy Sotelo, a visiting researcher in the Spring group. Professor Sotelo synthesised two alkyne-linked ciprofloxacin derivatives **90** and **91** (see Figure 22), both with cleavable linkers (see 5.3.7).

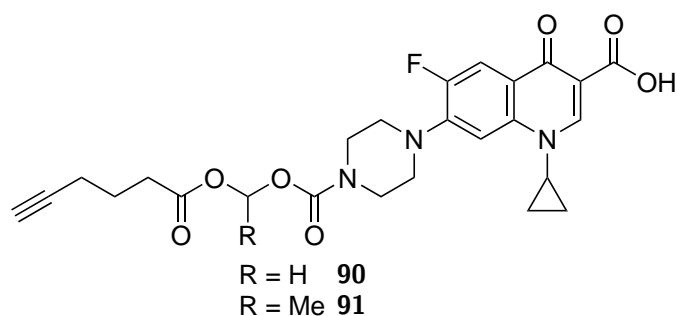


Figure 22: The cleavable alkyne-Cip derivatives synthesised by Professor Sotelo.

Professor Sotelo then performed click reactions using the AHL azide derivatives **55**, **58** and **61** synthesised in 7.2.3 to form a new set of conjugates to add to the library (see Figure 23). It was hoped that these conjugates would enter the cell and then be cleaved by esterases to release ciprofloxacin (see 5.3.7).

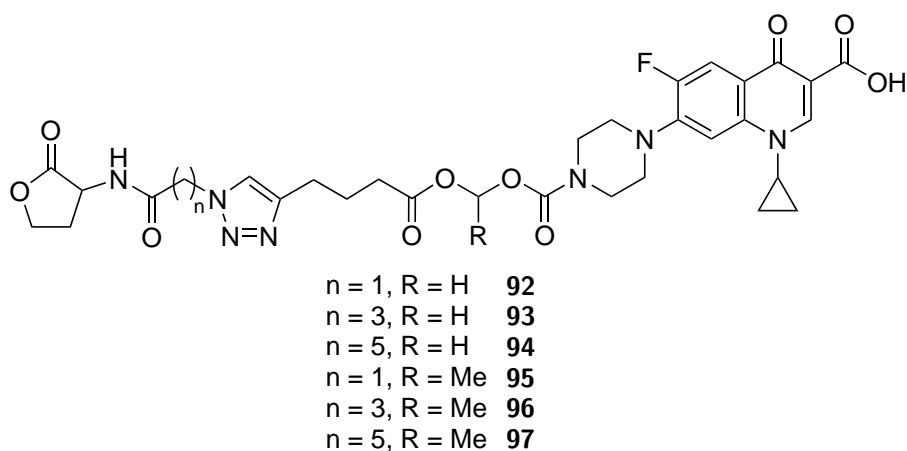


Figure 23: The cleavable HSL-Cip triazole conjugates synthesised by Professor Sotelo.

Two control compounds **98** and **99** with benzyl head groups were also produced by Professor Sotelo (see Figure 24). It was hoped that these would show whether the AHL head group is required for activity.

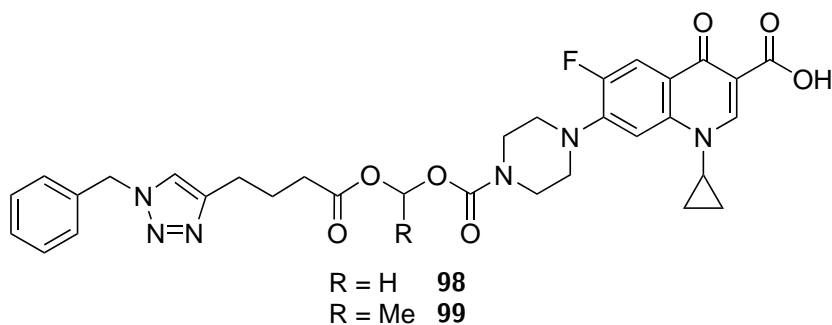


Figure 24: The cleavable Bn-Cip triazole conjugates **98** and **99** synthesised by Professor Sotelo.

## 7.5 Biological testing

### 7.5.1 Autoinducer-antibiotic conjugates

The eight triazoles made in 7.4 (see Figure 25) were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* PAO1<sup>180</sup> and YM64.<sup>181</sup> PAO1 is the *P. aeruginosa* wild-type strain. YM64 is a mutant lacking all of the four major *mex* operons for multidrug efflux pumps: *mexAB-oprM*, *mexXY*, *mexCD-oprJ* and *mexEF-oprN*, making it more sensitive to many antibiotics and hence able to show up moderate effects more clearly.

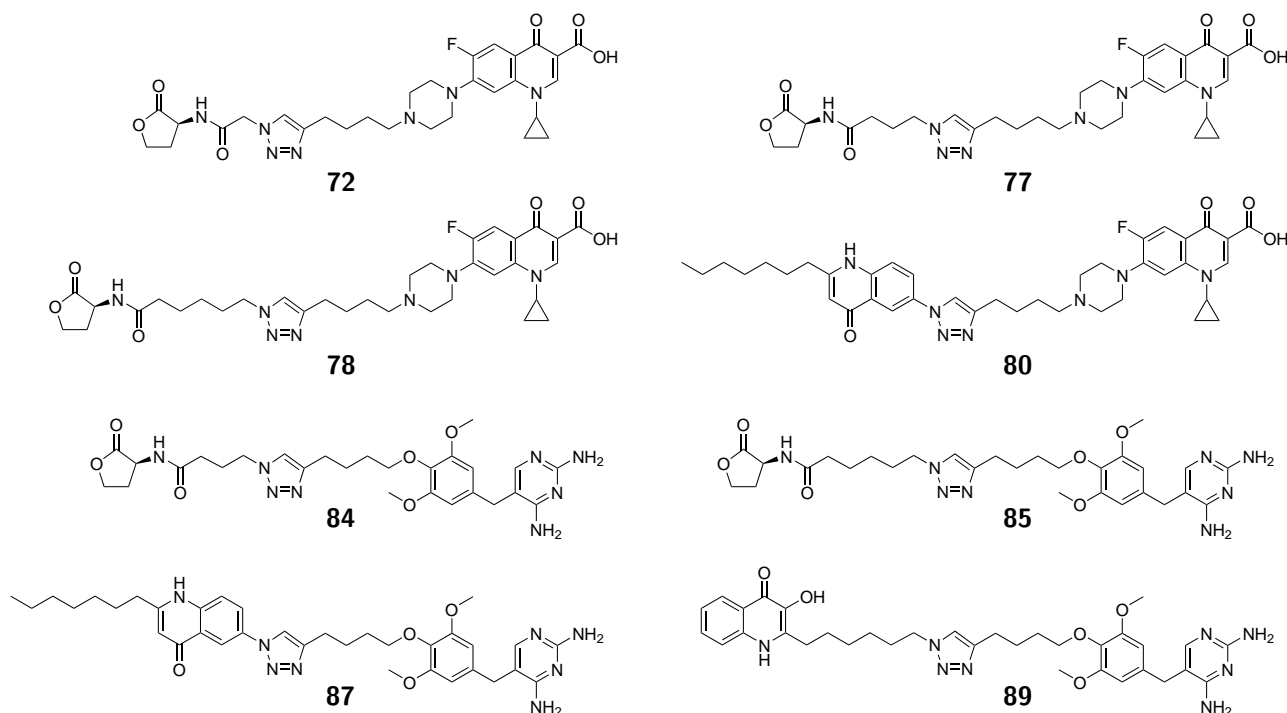


Figure 25: The autoinducer-antibiotic conjugates.

#### 7.5.1.1 Antibacterial and anti-biofilm testing against YM64

In YM64 at 5 h the HSL-Cip conjugates **72**, **77** and **78** showed slight activity at the highest concentration, but not as much as ciprofloxacin **24**. This activity was not visible by 24 h (see Figure 27) and the compounds had no effect on biofilm formation (see Figure 28).

A dose-dependant response was expected for these results, however this was not seen except for a slight effect at 5 h in YM64 for some compounds. The dose-dependant response might potentially be seen if higher concentrations were tested, although there could be problems with compound solubility. Conversely, the dose-dependant response might be seen for ciprofloxacin **24** if lower concentrations were tested. Smaller ‘steps’ in concentration could also be tested after establishing the range over which the dose-dependant response is seen.

The very high readings in the biofilm assays for ciprofloxacin **24** (see Figure 28 and Figure 31) could be due to sub-MIC concentrations of the antibiotic causing the bacteria to respond by forming a protective biofilm. This effect has been reported with cefotaxim, amoxicillin, azithromycin,<sup>182,183</sup> tobramycin, amikacin, streptomycin and gentamicin, but oddly not with ciprofloxacin **24**,<sup>184,185</sup> although this could be due to the exact conditions of growth.

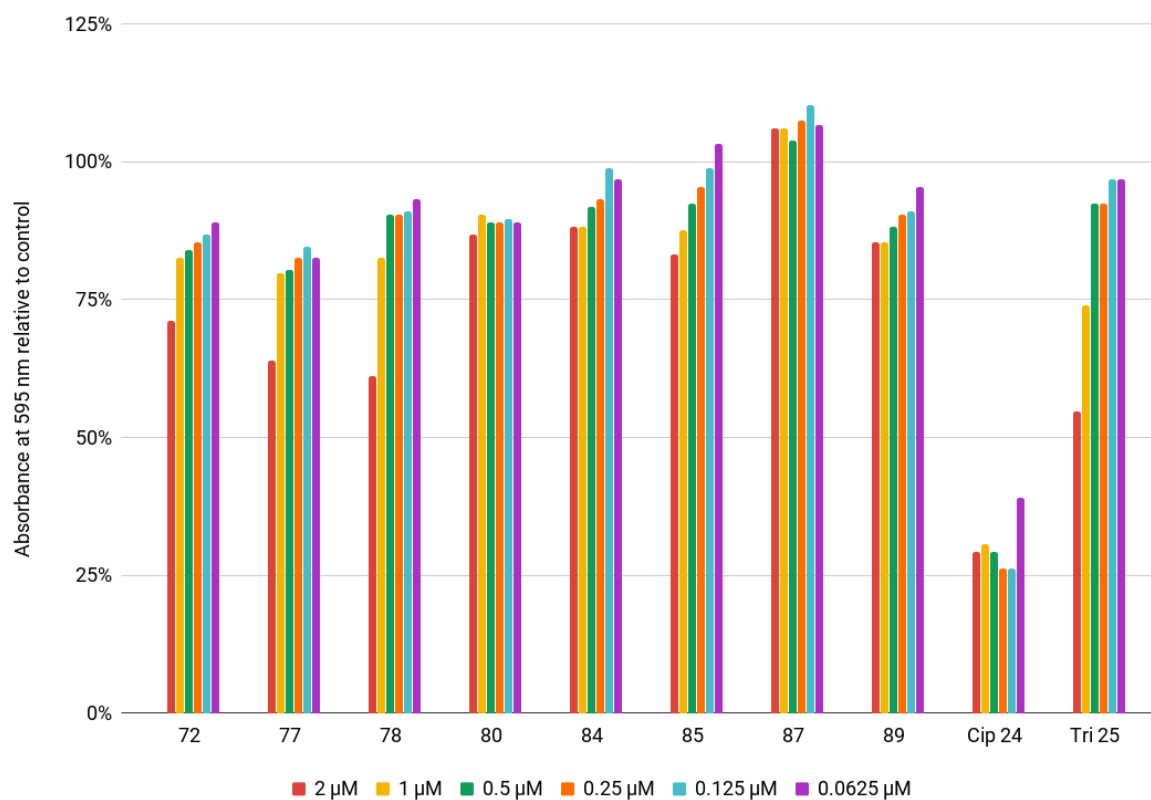


Figure 26: YM64 optical density (OD) readings at 5 h for the autoinducer-antibiotic conjugates.

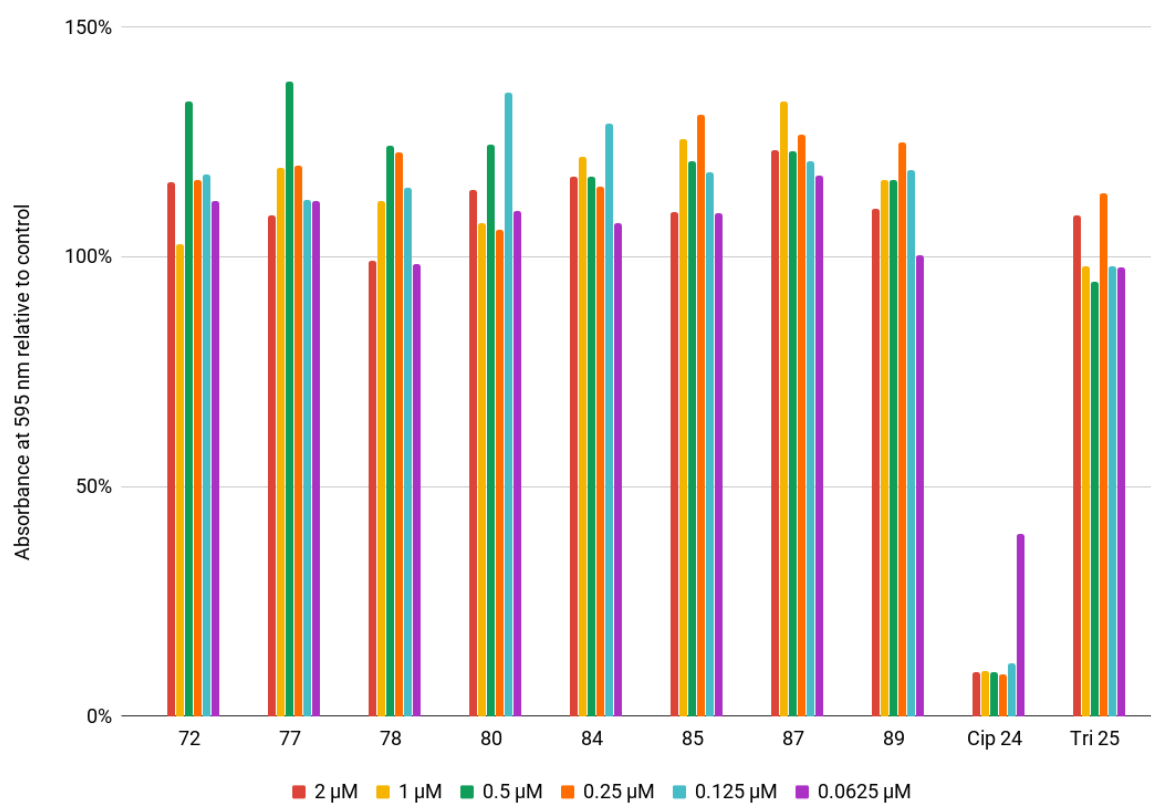


Figure 27: YM64 OD readings at 24 h for the autoinducer-antibiotic conjugates.

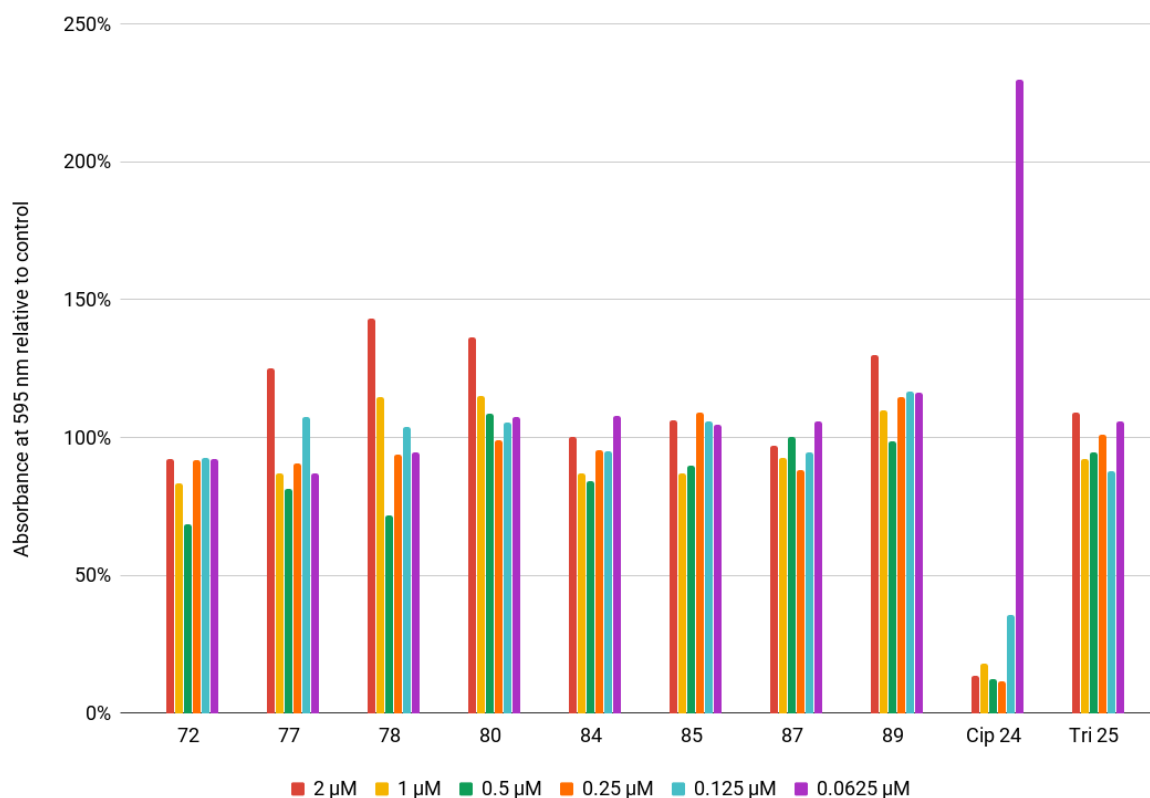


Figure 28: YM64 biofilm quantification at 24 h for the autoinducer-antibiotic conjugates.

#### 7.5.1.2 Antibacterial and anti-biofilm testing against PAO1

In PAO1 **78** showed similar activity to ciprofloxacin **24** at the highest concentration (see Figure 29), but not at lower concentrations. All other compounds did not show activity, and again there was no activity at 24 h or against biofilms. Increased biofilm formation is again seen with intermediate concentrations of ciprofloxacin **24**, although PAO1 seems to be overwhelmed at the highest concentrations.

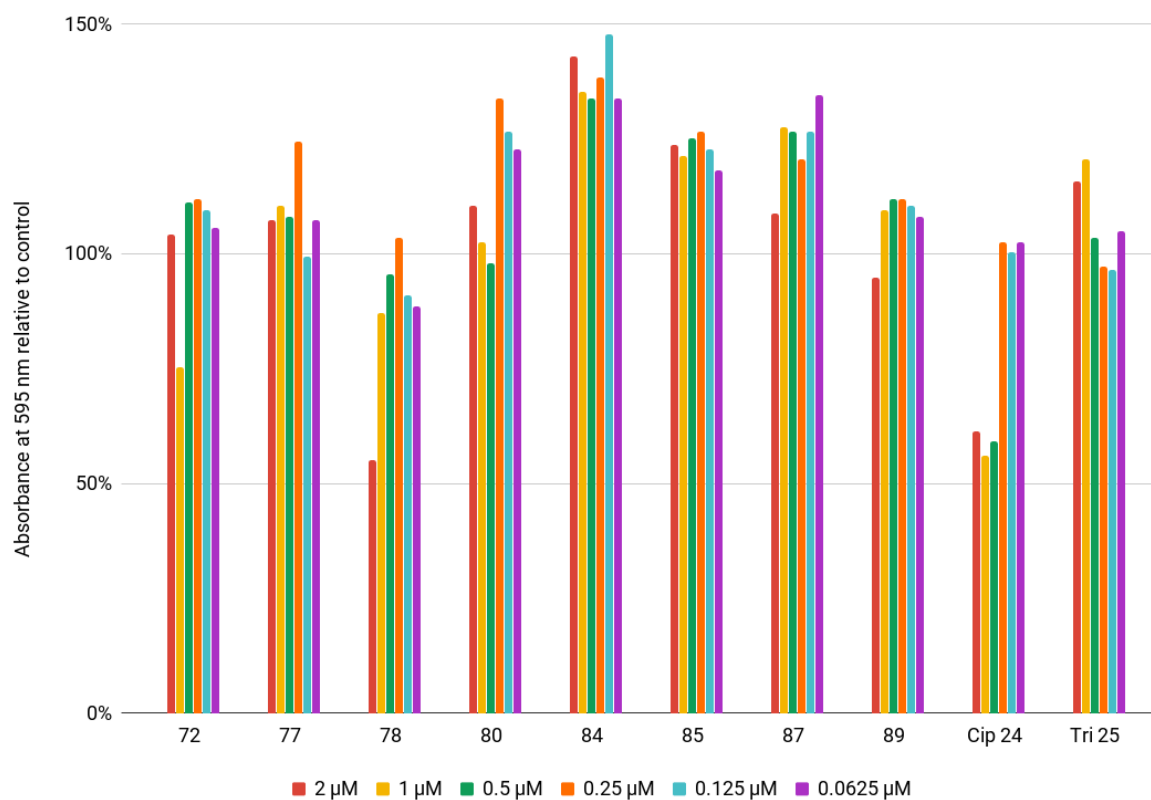


Figure 29: PAO1 OD readings at 5 h for the autoinducer-antibiotic conjugates.

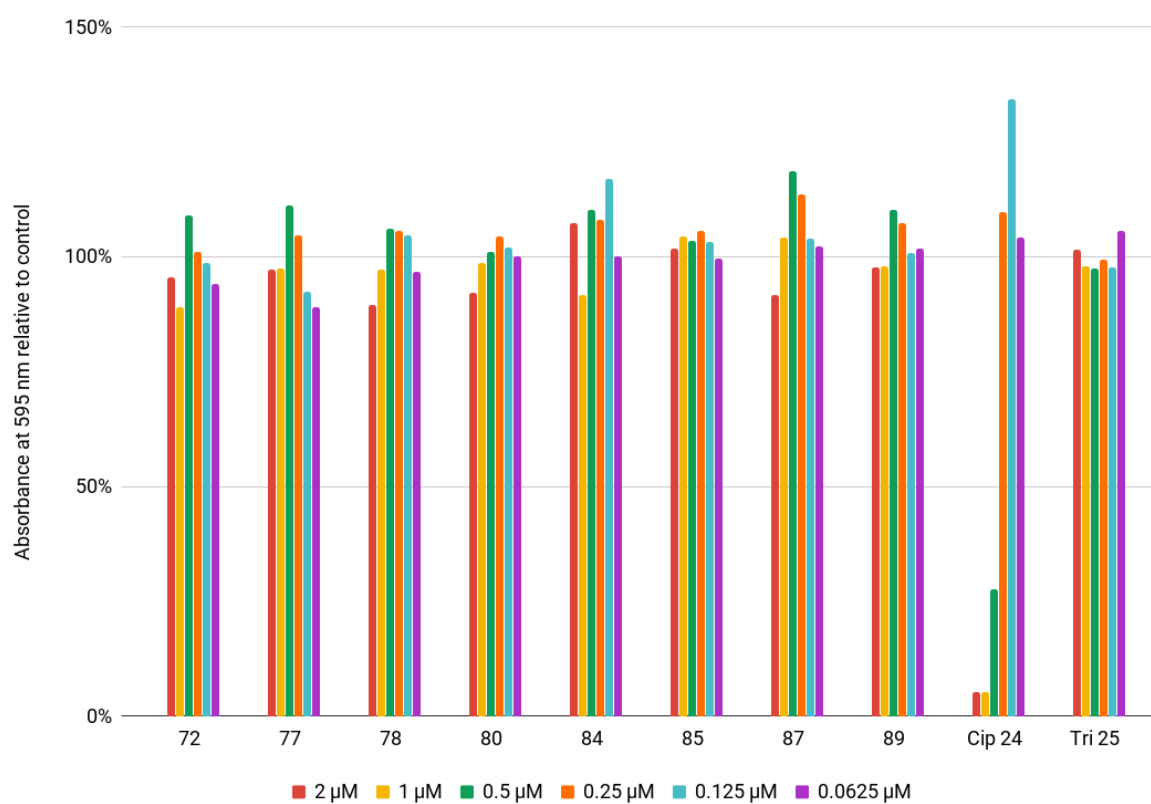


Figure 30: PAO1 OD readings at 24 h for the autoinducer-antibiotic conjugates.

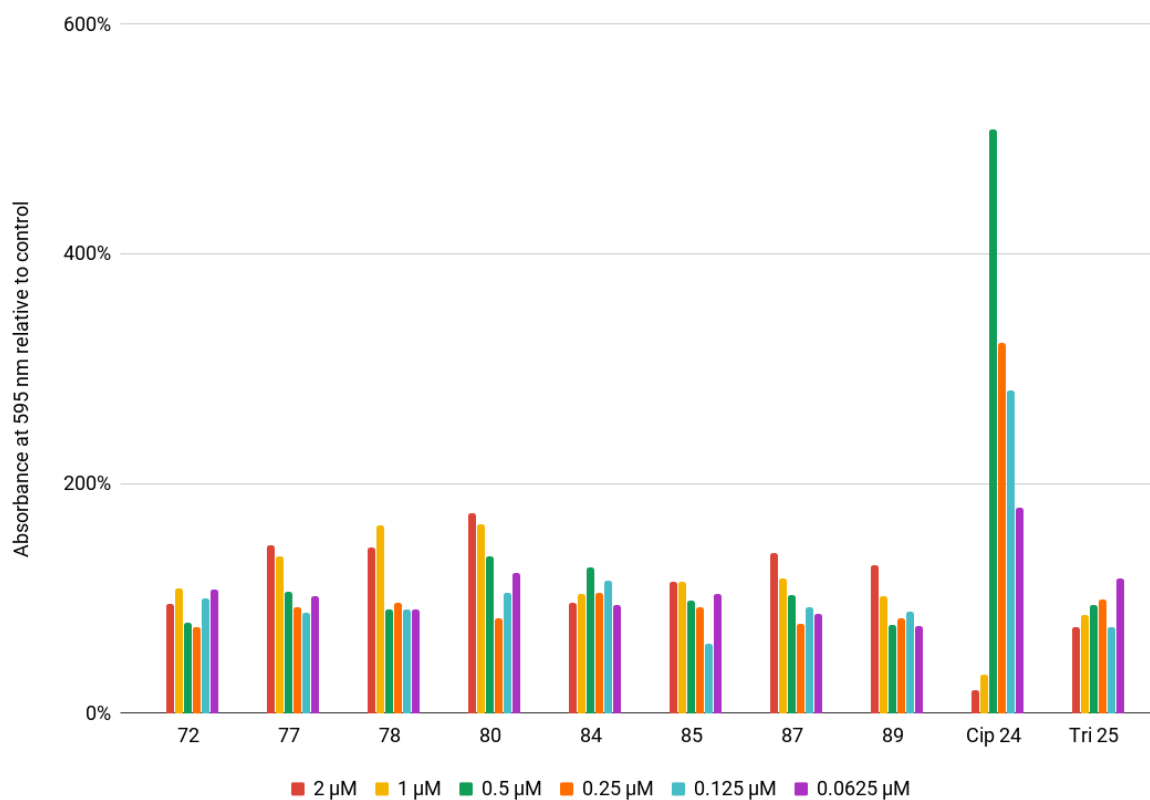


Figure 31: PAO1 biofilm quantification at 24 h for the autoinducer-antibiotic conjugates.

### 7.5.2 Cleavable homoserine lactone-ciprofloxacin conjugates

The eight cleavable HSL-Cip conjugates, two controls and two alkynes described in 7.4.3 (see Figure 32) were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* YM64.



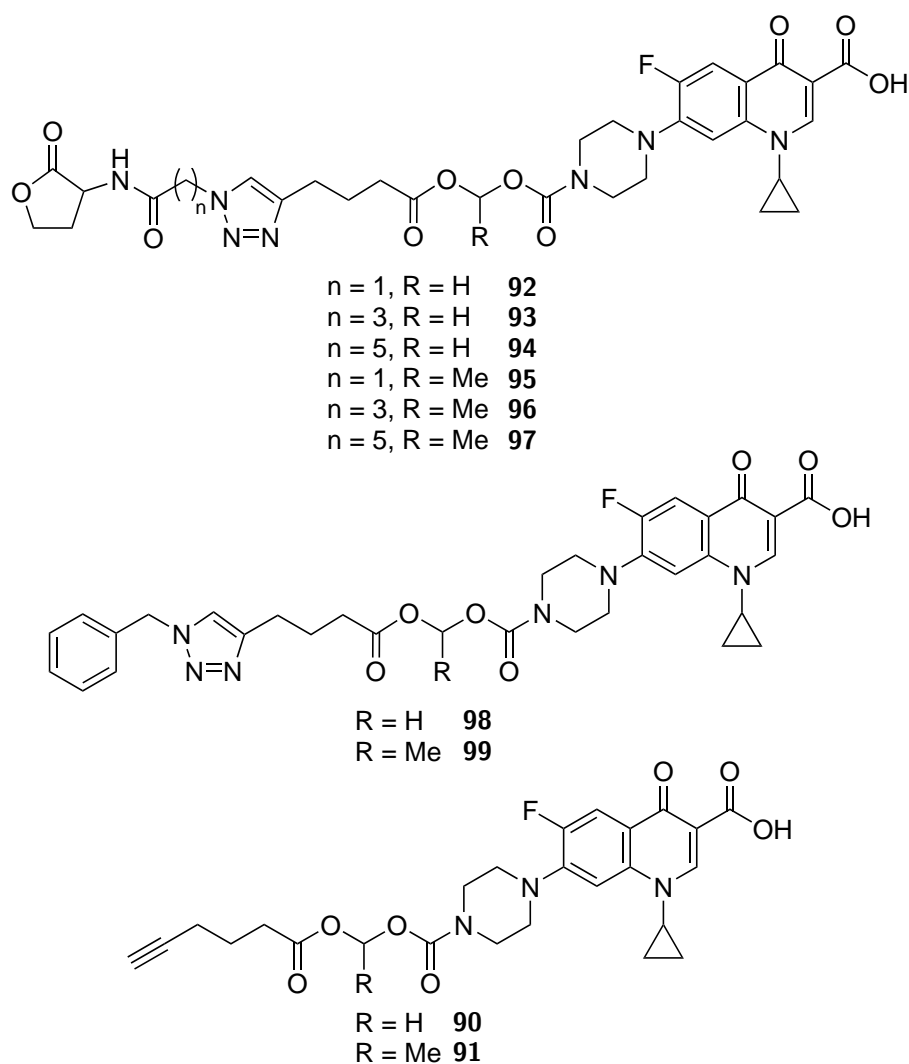


Figure 32: The cleavable HSL-Cip conjugates.

Here there was more success, although the activity was still not as high as for ciprofloxacin **24**. The HSL-Cip conjugates with *N*-(acetoxymethoxycarbonyl) linkers ( $R = H$ ) showed activity at high concentrations. A longer linker seems to give higher activity; **93** and **94** showed activity comparable with ciprofloxacin **24** at high concentrations. Unfortunately the control **98** and alkyne **90** with *N*-(acetoxymethoxycarbonyl) linkers ( $R = H$ ) showed higher activity than the conjugates, indicating that the HSL head wasn't contributing to the activity of the conjugates.

The conjugates with an *N*-(acetoxymethoxycarbonyl) linker ( $R = Me$ ) did not show any activity. This suggests that they either didn't enter cells or weren't suitable substrates for esterases. The *N*-(acetoxymethoxycarbonyl) linked alkyne ( $R = Me$ ) did show some activity, indicating that maybe it could penetrate cells more easily than the conjugates due to its lower molecular weight and/or lower polarity.

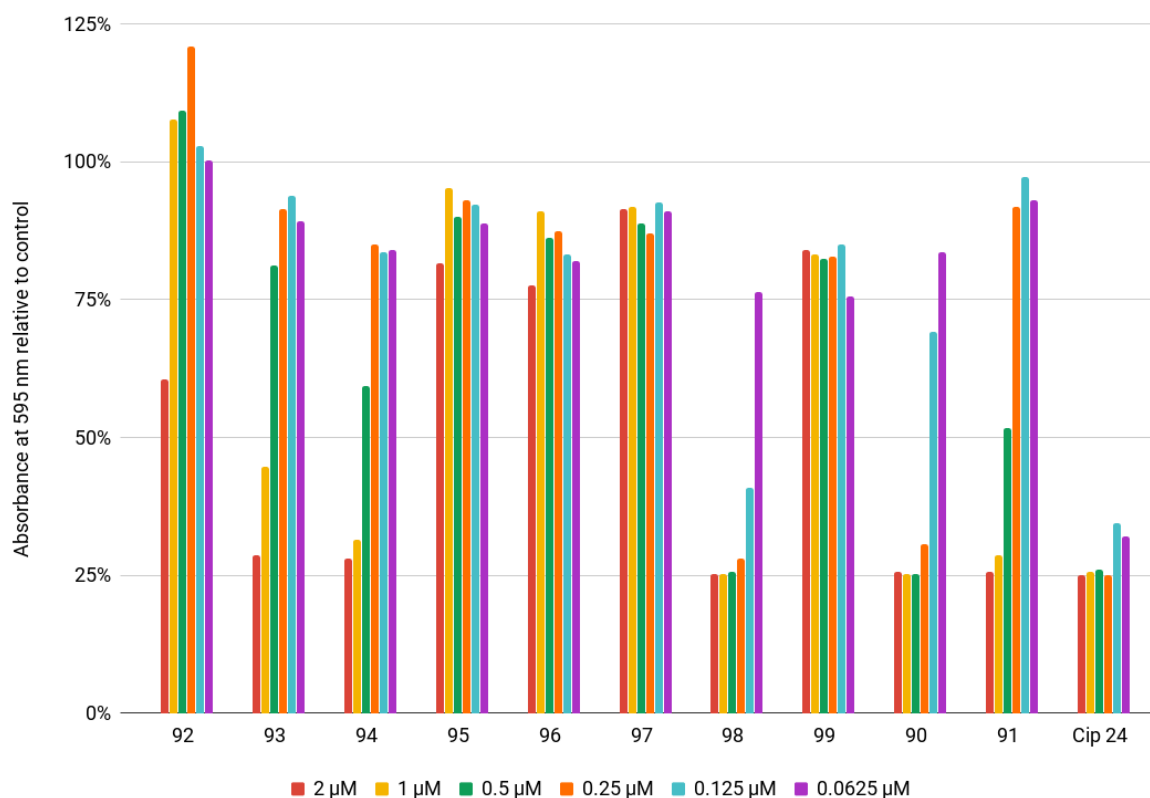


Figure 33: YM64 OD readings at 5 h for the cleavable HSL-Cip conjugates.

## 7.6 Conclusions

### 7.6.1 Library synthesis

In this section, a range of 1,2,3-triazole-linked autoinducer-antibiotic conjugates was successfully synthesised and tested for antibiotic and anti-biofilm activity. Reliable routes to the azido autoinducers and alkynyl antibiotics were found, but the copper(I)-catalyzed alkyne-azide cycloaddition reactions used to link them proved rather capricious. The main reasons for this were insolubility of the starting materials and air-sensitivity. Air-sensitivity is not expected in a click reaction, but can be explained by many of the reactions being too dilute.<sup>186</sup> This led to ascorbate being used up by the oxygen dissolved in the reaction solvent and present in the air above the reaction mixture. Even when the solvent was degassed and the reaction performed under argon, a small amount of air leaking in through a perished septum was enough to cause the reaction to stall. Low concentrations were used because of the insolubility of the starting materials, but this would have been better addressed by more thorough screening of solvents. In addition, it was later shown that THPTA may not be necessary for a sufficiently concentrated reaction to take place,<sup>187</sup> so this expensive reagent could be omitted.

Assuming the click reaction could be further optimised, this library could be easily expanded by the addition of more azido autoinducers and alkynyl antibiotics (see 7.7). In particular, autoinducers which are actively transported into cells, such as AI-2, are attractive targets.

### 7.6.2 Biology

Little biological activity was seen in the non-cleavable autoinducer-antibiotic conjugates. This could be due to a number of factors, including:

1. Restriction of the binding of ciprofloxacin **24** to DNA gyrase and topoisomerase IV<sup>135</sup> or trimethoprim

**25** to dihydrofolate reductase.<sup>136</sup> This could be investigated by measuring binding of the compounds to the purified protein targets.

2. Failure to penetrate the cell wall/biofilm or non-specific binding to the cell wall. This could be investigated by separation of the cultures by centrifugation and quantification of the compounds in each fraction by HPLC.
3. Failure of the autoinducers to mask the antibiotics from recognition by efflux pumps. While the resistance of YM64 to the conjugates suggests that this mechanism is unlikely, YM64 is not lacking every efflux pump,<sup>8,181</sup> so it is possible that the conjugates are still being pumped out. A strain with all pumps knocked out, or a suitable cocktail of pump inhibitors, would be needed to investigate this mechanism fully.

If binding of the antibiotics to target proteins is indeed restricted by the attachment of the autoinducer, this could be affected by the size and polarity of the linker and autoinducer. With this in mind, the next set of compounds synthesised contain HSL analogues, which are smaller than HHQ **21** and PQS **22**, and some omit the triazole in the linker, hence affecting polarity.

The cleavable HSL-Cip conjugates showed a little more activity, but unfortunately this did not require the HSL, and probably was mostly affected by the polarity and size of the attached group and the ease of hydrolysis of the linker.

## 7.7 Future work

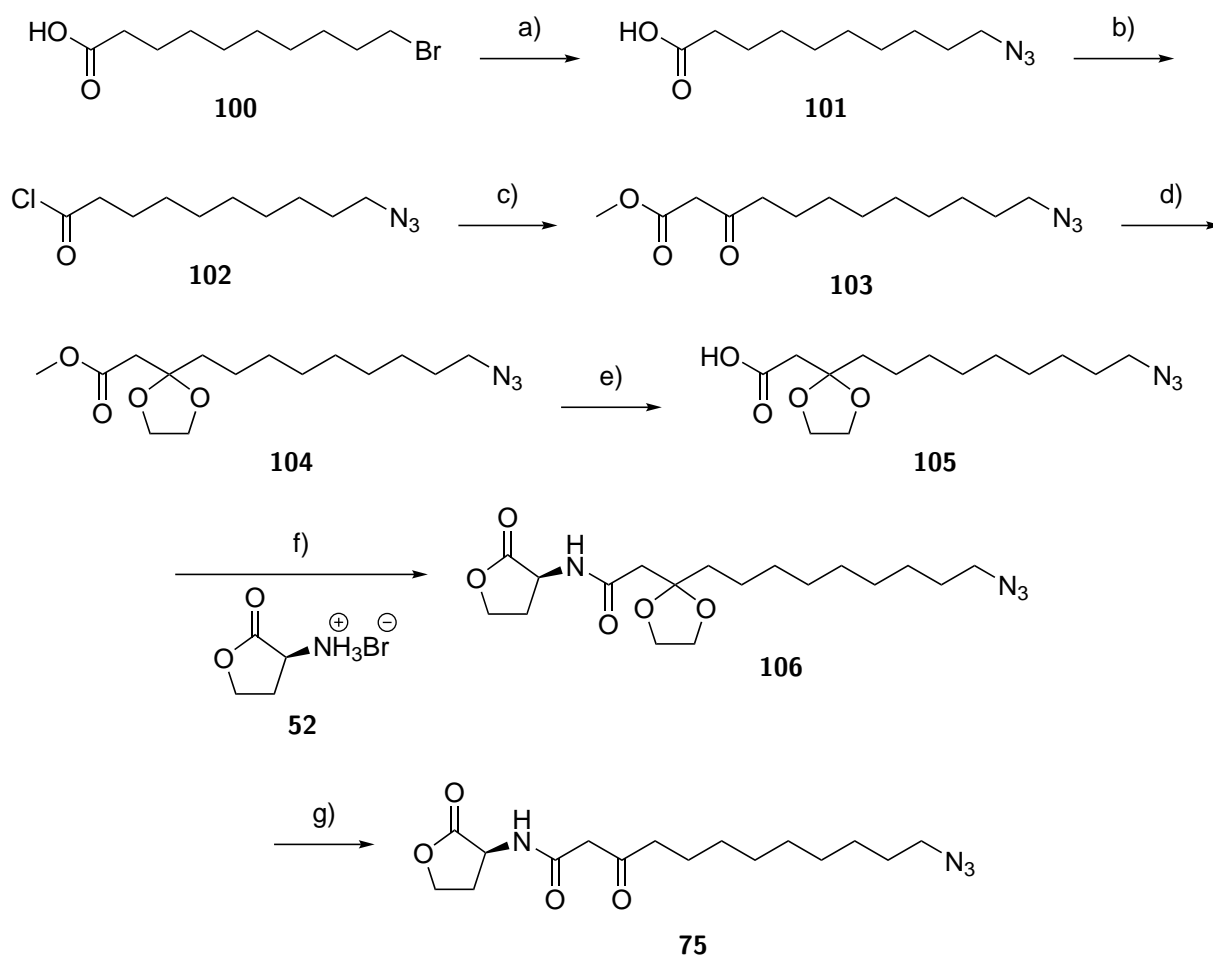
This section begins with discussion of further autoinducers and antibiotics which could be used in future conjugates. Some have already been partially or fully synthesised by myself or other members of the Spring group. Plans for further biological testing of the conjugates synthesised in this study are then presented.

### 7.7.1 Autoinducer derivatives

#### 7.7.1.1 3-oxo-C<sub>12</sub>-HSL derivative **75**

N<sub>3</sub>-3-oxo-C<sub>12</sub>-HSL **75** (see Scheme 11) was synthesised by Ryan Howard, a master's student under my supervision. The synthesis was based on a synthesis of 3-oxo-C<sub>12</sub>-HSL **20** reported by Hodgkinson *et al.*<sup>89</sup> Conjugates of this compound were not included in the library as it degraded during the click reaction. However, reaction conditions could be further optimised, or the acetal-protected azide **106** could be used in the click reaction, followed by deprotection.

This compound would be a useful addition to the library as it would demonstrate whether the 3-oxo group and/or longer alkyl chain are required for activity. As the head group is added fairly late in the synthesis it would also be easy to swap it for the other head groups described in 8, thus expanding the library further.



Scheme 11: The synthesis of N<sub>3</sub>-3-oxo-C<sub>12</sub>-HSL **75** carried out by Ryan Howard. a) NaN<sub>3</sub>, DMF, 60 °C, 6 h, 93%. b) Oxalyl chloride, DMF, CH<sub>2</sub>Cl<sub>2</sub>, 3 h, r.t.. c) MeOAc, *N*-methyl imidazole, TiCl<sub>4</sub>, DIPEA, toluene, r.t., 2 h, 43% over two steps. d) HO(CH<sub>2</sub>)<sub>2</sub>OH, TsOH, CH(OMe)<sub>3</sub>, r.t., 5 h, 78%. e) NaOH, water, r.t., 6 h, 85%. f) EDC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 16 h. g) TFA, r.t., 5 h, 29% over two steps.

#### 7.7.1.2 AI-2 derivatives

AI-2 **23** is perhaps a more attractive choice of autoinducer for inclusion in conjugates than the others used in this study as it is actively transported into cells<sup>188</sup> and used by a wide range of bacterial species.<sup>125</sup> The synthesis of conjugates of AI-2 **23** with ciprofloxacin **24** and trimethoprim **25** has been attempted in the Spring group by Dr Jamie Stokes. However, the protected azido AI-2 derivative **107** synthesised was found to be unstable, and the click reactions attempted were unsuccessful.<sup>187</sup> AI-2 **23** is known to interconvert between multiple forms (including forming a furanosyl borate diester)<sup>189</sup> so it is to be expected that syntheses involving it might be challenging. If a more stable azido AI-2 derivative cannot be developed, another approach would be to use an azido AI-2 analogue which is capable of being taken up by the same active transport mechanism.

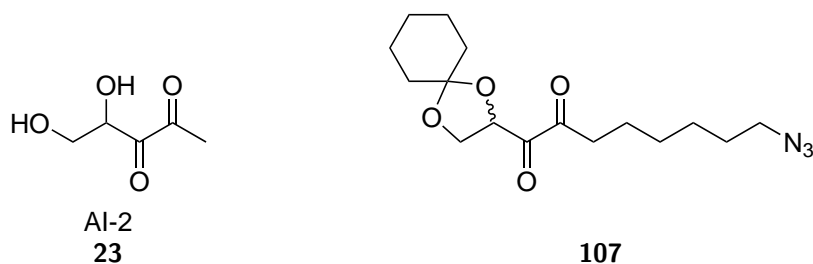


Figure 34: AI-2 **23** in its DPD form and the protected azido AI-2 derivative **107** synthesised by Dr Jamie Stokes.<sup>187</sup>

Two types of AI-2 **23** receptors have been identified: LuxP, present in *Vibrio* spp.,<sup>190</sup> and LsrB, first discovered in *Salmonella enterica* serovar Typhimurium.<sup>191</sup> LuxP is a periplasmic binding protein that relays the signal, but not the actual AI-2 **23** molecule, into the cell and hence is not a useful target.<sup>192</sup> LsrB is the ligand binding protein of a system that transports AI-2 **23** into the cell,<sup>188</sup> and hence can be targeted. LsrB orthologs are found in a wide range of bacterial families including *Enterobacteriaceae*, *Rhizobiaceae*, and *Bacillaceae*.<sup>193</sup> In addition, several bacterial species, including *P. aeruginosa*, are known to respond to AI-2 **23** but do not have either of these two known types of receptors, and thus the discovery of new receptor types is expected.<sup>193</sup> Any postulated receptor would need to internalise the AI-2 analogue in order for conjugates to be effective against the bacterium.

One example of an AI-2 analogue which could be derivatised is a geminal dibromo compound **108** synthesised by Guo *et al.*<sup>189</sup> (see Figure 35). It is as potent as AI-2 at dissociating the LsrR repressor from the promotor region in a reporter strain, and may be more stable. It is also esterified, making it less volatile and thus easily purified using column chromatography. The esters are presumably cleaved by cellular esterases as the compounds can be used in QS assays without deprotection.<sup>194</sup>

A possible azido derivative **109** of this analogue is shown in Figure 35. If a route to it could be found, it appears to be a promising partner in future conjugates given the known properties of AI-2 **23**.

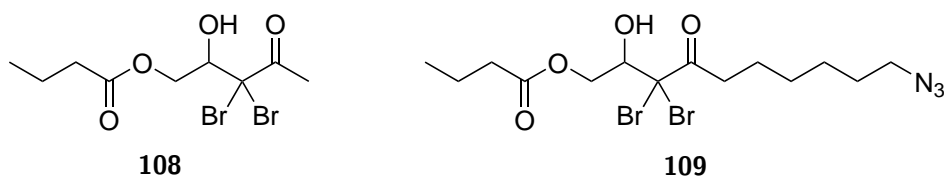


Figure 35: An AI-2 analogue **108** synthesised by Guo *et al.* and the proposed azido AI-2 analogue derivative **109**.

## 7.7.2 Antibiotic derivatives

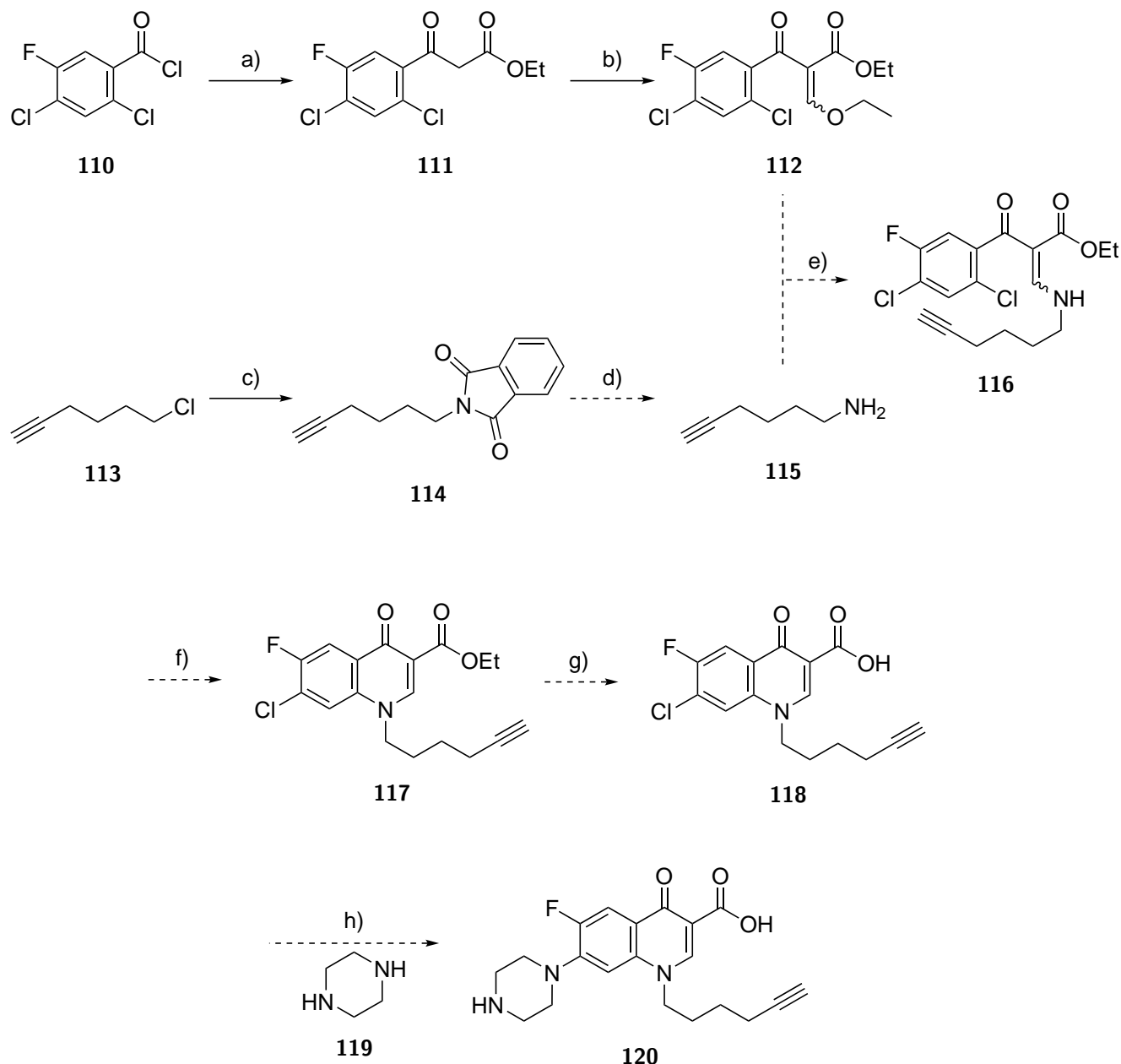
### 7.7.2.1 Ciprofloxacin derivative **120**

A second alkynyl ciprofloxacin derivative **120** was planned and partially synthesised during this project, and finishing this synthesis would provide a useful intermediate for future conjugates.

The derivative **120** has an alkyne tail attached in place of the cyclopropane ring as it has been shown that bulkier groups in this position can be tolerated.<sup>195, 196</sup> This synthesis followed a conventional route to ciprofloxacin **24** similar to that reported by Mitscher *et al.*<sup>195</sup> but used hex-5-yn-1-amine **115** instead of cyclopropylamine.

The TiCl<sub>4</sub>-catalysed crossed Claisen condensation of the acid chloride **110** and ethyl acetate described by Hashimoto *et al.*<sup>197</sup> was used to produce the  $\beta$ -ketoester. The ethoxymethylene group in **112** was installed by

the reaction of  $\beta$ -ketoester **111** and triethyl orthoformate to give a mixture of the *E* and *Z* isomers.<sup>195, 198</sup> Hex-5-yn-1-amine **115** was prepared using a Gabriel synthesis<sup>199</sup> described by Rożkiewicz *et al.*<sup>200</sup> Unfortunately the amine was surprisingly volatile and was lost on evaporation of the reaction solvent. If a better purification method could be found, or a longer-chain alkynyl amine was used, the rest of the synthesis could be performed and the resulting alkynyl ciprofloxacin derivative **120** could be used to form more triazole-linked conjugates.



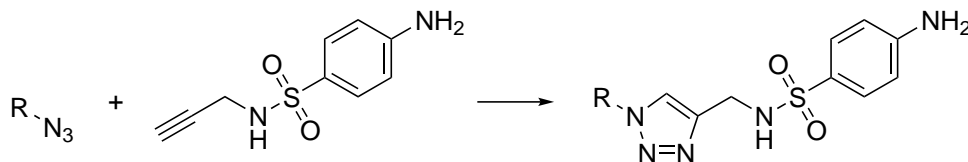
Scheme 12: The synthesis of **120**. a) EtOAc, TiCl<sub>4</sub>, DIPEA, *N*-methyl imidazole, toluene, r.t., 30 min. b) Triethyl orthoformate, Ac<sub>2</sub>O, reflux, 2 h. c) Potassium phthalimide, potassium iodide, DMF, 80 °C, 18 h. d) N<sub>2</sub>H<sub>2</sub> · H<sub>2</sub>O, EtOH, reflux, 18 h. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) DMSO.

### 7.7.2.2 Sulfanilamide derivatives

Sulfanilamide antibiotics were the first class of antibiotics to be widely used.<sup>201, 202</sup> They are all derivatives of 4-aminobenzenesulfonamide, very commonly with the sulfonamide nitrogen linking to a heterocycle. Sulfanilamide antibiotics function by inhibiting bacterial synthesis of folic acid. *P. aeruginosa* has intrinsic resistance to sulfanilamides mainly due to the MexAB-OprM efflux pump<sup>140</sup> and so, as with trimethoprim **25**, it is hoped

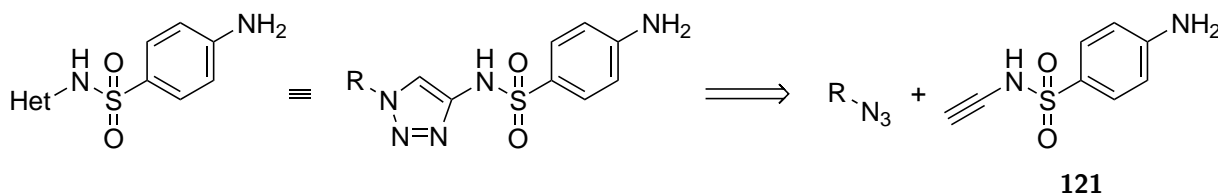
that conjugation to an autoinducer would restore activity.

Derivatives of 4-aminobenzenesulfonamide **216** have previously been synthesised using copper(I)-catalyzed alkyne-azide cycloaddition reactions to append various groups<sup>203</sup> (see Scheme 13). However, if one considers sulfonamide antibiotics already in use, nearly all have a heterocycle linked directly to the sulfur atom, rather than with a methylene group in between.



Scheme 13: The sulfanilamide derivatives synthesised using click chemistry by Wang et al.<sup>203</sup>

Therefore, it was postulated that a 1,2,3-triazole could be introduced in the position occupied by a heterocycle in other known sulfonamide antibiotics by attachment of an alkyne directly to the sulfonamide nitrogen to form an alkynyl sulfanilamide derivative **121** or a protected version of it (see Scheme 14).

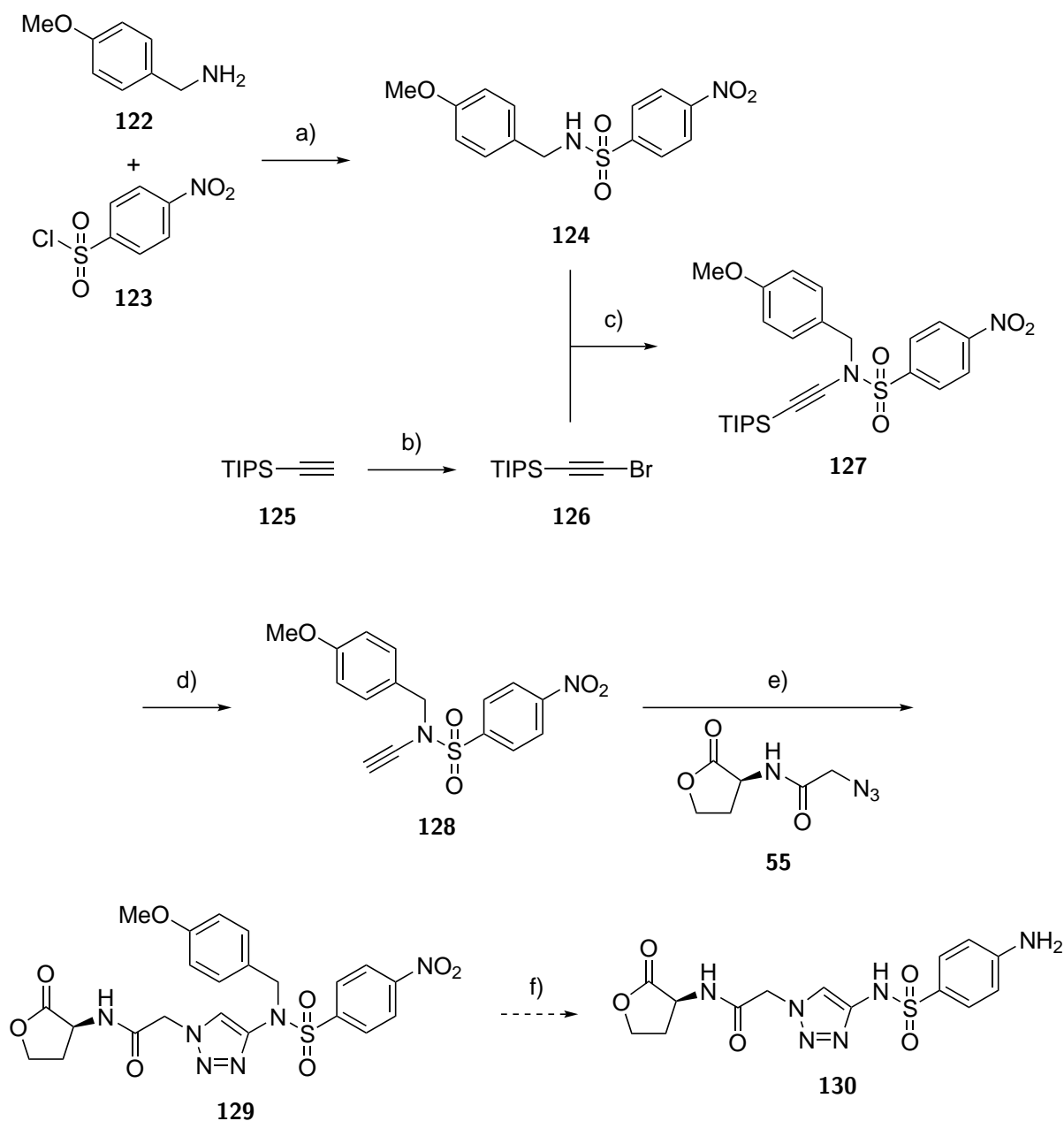


Scheme 14: Retrosynthesis of a 1,2,3-triazole-containing autoinducer-sulfonamide conjugate. R = autoinducer.

It was hoped that sulfanilamide derivative **121** could be synthesised and reacted with the azido autoinducer derivatives directly. However, it appears that no secondary ynamides have been synthesised to date. Conversely, the synthesis of tertiary ynamines has been studied more widely.<sup>204</sup> In particular, tertiary ynamides have been shown to be relatively stable and easy to work with in a variety of reactions including copper(I)-catalyzed alkyne-azide cycloadditions.<sup>205, 206</sup>

The study of copper(I)-catalyzed alkyne-azide cycloadditions of ynamides by IJsselstijn et al.<sup>205</sup> includes terminal ynamides protected using a benzyl and a tosyl group. Although their click reactions proceed with high yield, they do not present the deprotection of their final compounds. However, these reactions provided a promising suggestion that click reactions between a protected alkynyl sulfanilamide derivative and the azido autoinducer derivatives are feasible. The tosyl group used by IJsselstijn et al.<sup>205</sup> to protect their ynamide is very similar to the *p*-aminobenzenesulfonyl group needed in the alkynyl-sulfanilamide derivative. However, because installation of the alkyne could be problematic in the presence of a second amine, the  $NH_2$  group was installed as a  $NO_2$  group and reduced after the click reaction.

The synthesis proceeded as shown in Scheme 15.<sup>205, 207, 208</sup> It was hoped that the methoxybenzyl group could be removed and the nitro group converted to an amine simultaneously by reduction in the last step, but unfortunately the methoxybenzyl group proved difficult to remove. On reflection, methoxybenzene was a poor choice of protecting group, and a more reduction-labile group such as benzyl or diphenylmethyl should have been chosen.<sup>209</sup> This reaction could be repeated with a different choice of protecting group to provide another set of autoinducer-antibiotic conjugates.



Scheme 15: Synthesis of a 1,2,3-triazole-containing sulfonamide antibiotic-autoinducer hybrid. a)  $\text{CH}_2\text{Cl}_2$ , r.t., 24 h. b)  $\text{AgNO}_3$ , acetone, r.t., 3 h. c)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 1,10-phenanthroline,  $\text{K}_2\text{CO}_3$ , toluene,  $80^\circ\text{C}$ , 48 h. d) TBAF, THF,  $-78^\circ\text{C}$ , 3 h. e)  $\text{Cu}(\text{OAc})_2$ , sodium ascorbate,  $\text{CH}_2\text{Cl}_2$ , *t*-BuOH, water, r.t., 16 h. f)  $\text{H}_2$ ,  $\text{PtO}_2$ , MeOH, 1 atm, r.t., 3 h.

### 7.7.2.3 Linezolid derivative 144

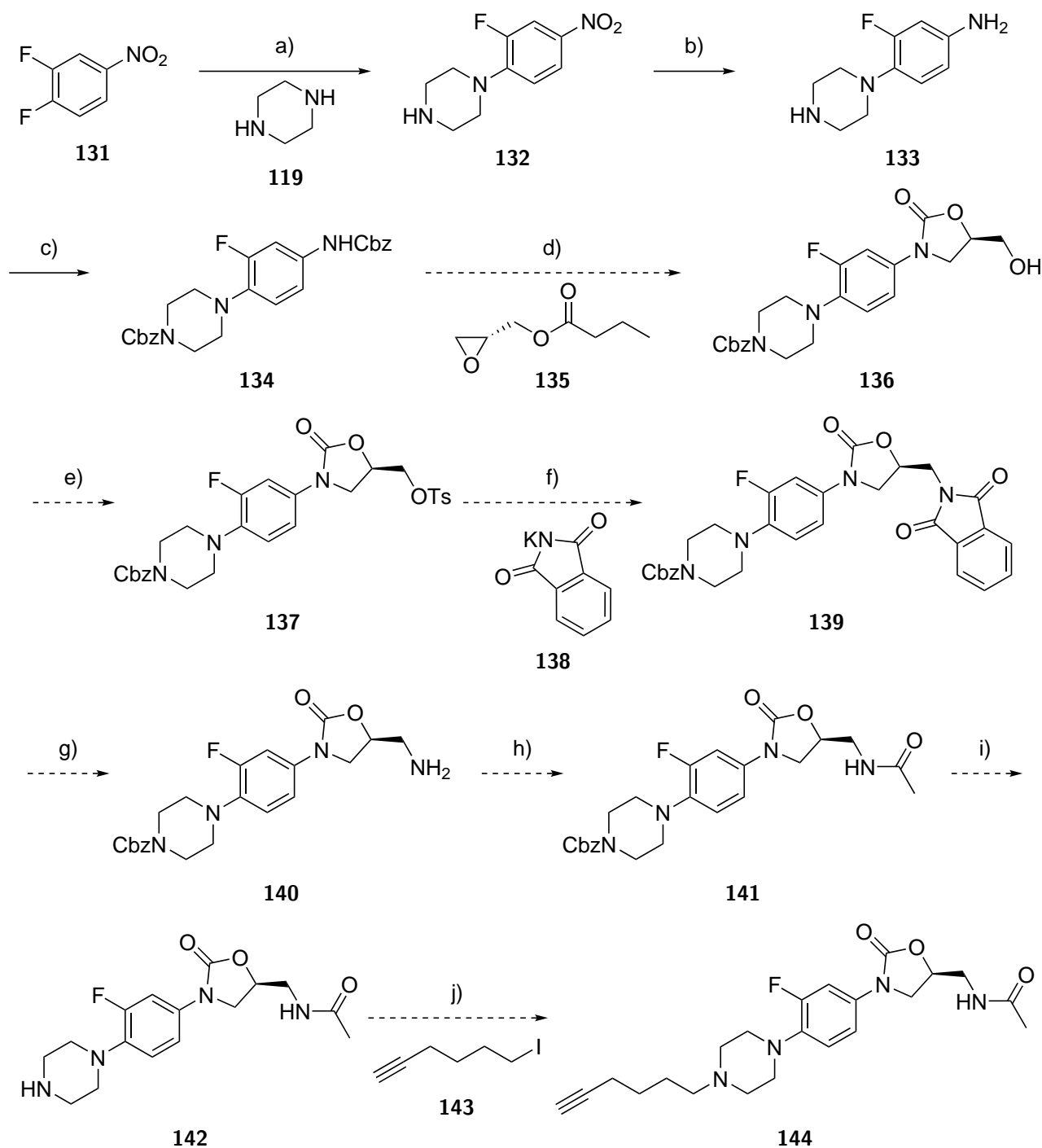
Linezolid is a monoamine oxidase inhibitor used for the treatment of infections caused by Gram-positive bacteria. Gram-negative bacteria, including *P. aeruginosa* are resistant to linezolid due to the activity of efflux pumps, and hence it might be possible to increase its activity in such organisms by increasing its uptake and/or retention by conjugation to an autoinducer.

An alkynyl linezolid derivative **217** was partially synthesised by Ryan Howard (see Scheme 16). The route follows a procedure described by Phetsang *et al*<sup>210</sup> where the morpholine ring of linezolid is replaced by piperazine, allowing an alkynyl tail to be attached to the molecule.

The first three steps were carried out on a large scale, producing 55.7 g of **134**. As all steps except the final one are reported in the literature<sup>210,211</sup> it is hoped that the alkynyl linezolid derivative **218** could be synthesised



fairly straightforwardly.



Scheme 16: Proposed and partially completed synthesis of linezolid derivative **218**.<sup>210</sup> a) MeCN, reflux, 3 h, 91%. b) H<sub>2</sub>, 10% Pd/C, THF, 40 psi, <50 °C, 1.5 h, 95%. c) CbzCl, Na<sub>2</sub>CO<sub>3</sub>, acetone, water, 5 °C, 1 h then r.t., 16 h, 56%. d) *n*-BuLi, THF, -78 °C, 1 h then add epoxide then -78 °C to r.t., 5 h. e) TsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to r.t. 4.5 h. f) Acetonitrile, water, reflux, 48 h. g) MeNH<sub>2</sub>, EtOH, water, reflux, 5.5 h. h) Ac<sub>2</sub>O, pyridine, 0 °C to r.t., 16 h. i) H<sub>2</sub>, 10% Pd/C, MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1 atm, r.t., 16 h. j) NEt<sub>3</sub>, EtOH, reflux.

#### 7.7.2.4 Gentamicin derivative 147

Gentamicin is an aminoglycoside antibiotic used to treat many bacterial infections, particularly those caused by Gram-negative organisms, by binding to the bacterial ribosome. Gentamicin is actually a mixture of components (see Figure 36) synthesised by *Micromonospora*, a genus of Gram-positive bacteria. Separation of the gentamicin

components has been achieved by Grote *et al.*<sup>212</sup> by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a **146** was isolated pure, and is particularly useful because it is the only component which contains a CH<sub>2</sub>NH<sub>2</sub> group. This group is less hindered than all other amine groups in gentamicin C1a **146** and hence it is possible to selectively derivatise the molecule at this position. Grote *et al.* attached a tag needed for an immunoassay using a pentafluorophenyl ester.<sup>213</sup> Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid **145** (see Scheme 17). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester **145** and then separate out the desired component.

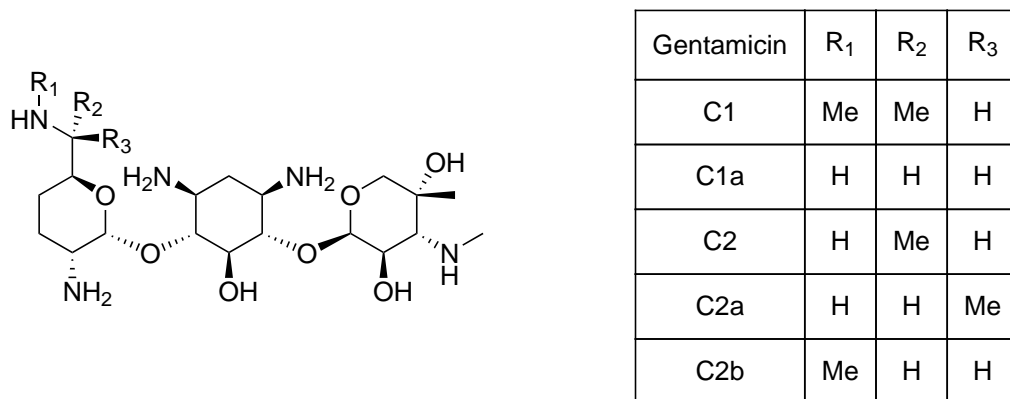
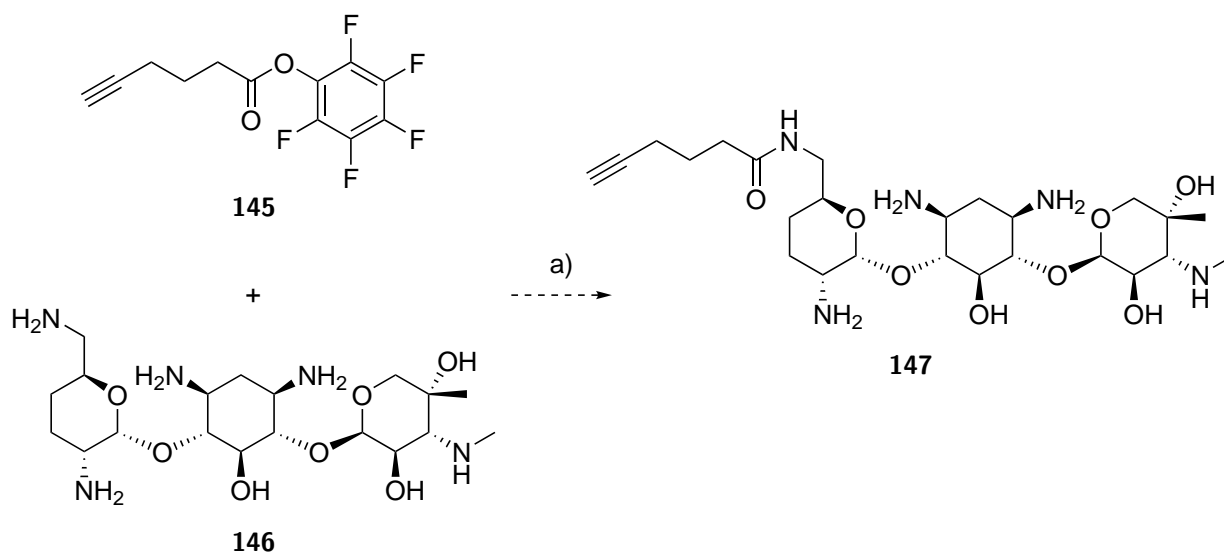


Figure 36: Gentamicin components.

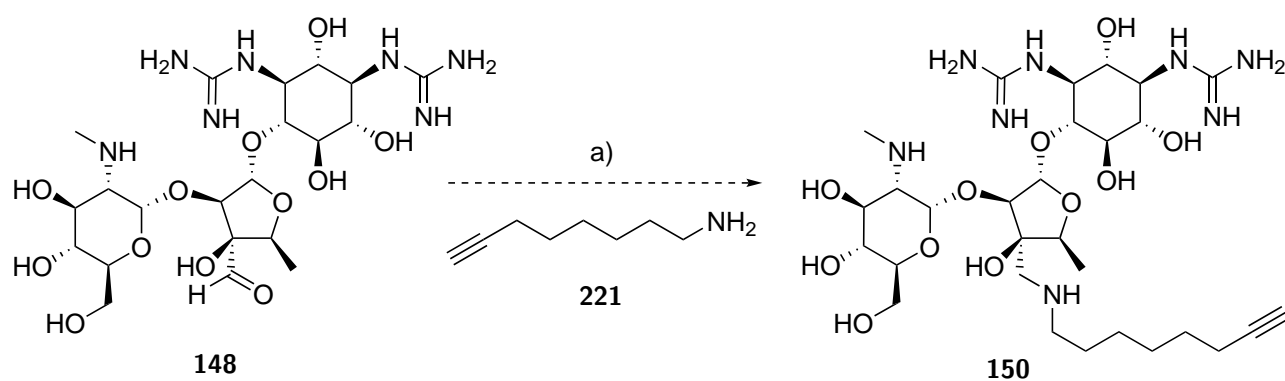


Scheme 17: Proposed synthesis of gentamicin C1a derivative **147**. a) DIPEA, DMF, - 55 °C.

#### 7.7.2.5 Streptomycin derivative **150**

Streptomycin **148** is an aminoglycoside antibiotic used to treat *Mycobacterium tuberculosis* and *S. aureus* which works by binding to the bacterial ribosome. There is limited SAR data on streptomycin but it is known that conversion of the aldehyde to a carboxylic acid destroys activity, whereas conversion to an alcohol retains it.<sup>214</sup>

Reductive amination can be used to install an alkyne group by reaction of the aldehyde with an amine such as oct-7-yn-1-amine **219** (see Scheme 18). This approach has been used by Zhang *et al.*<sup>215</sup> to form a conjugate of streptomycin **148** and chitosan which was active against biofilms. Reductive amination replaces the aldehyde O with NH; it is known that an OH is tolerated at this position so it makes sense that NH is as well.



Scheme 18: Proposed synthesis of streptomycin derivative **150**. a)  $\text{NaBH}_3\text{CN}$ , water, r.t..

### 7.7.3 Biology

The following extra biological data are required for these compounds:

1. Repeats of the antibacterial and anti-biofilm assays in order to assess variability in the data.
2. Growth curves to 48 h and biofilm quantifications for the cleavable HSL-Cip conjugates.
3. Biofilm dispersal assays on all compounds (see 5.3.8 for a discussion of biofilm dispersal using a HSL analogue-CipMe conjugate and 9.71.4 for the methodology to be used).

## 8 Results and discussion: homoserine lactone analogue-ciprofloxacin conjugates

### 8.1 Overview

The second part of this project was focused on producing a library of HSL analogue-Cip and -CipMe conjugates. The HSL head group was replaced with a selection of cyclic amines found in known quorum sensing modulators (see 5.3.8). The analogues were linked to ciprofloxacin **24** in two ways: directly using either an  $S_N2$  reaction or peptide coupling with methyl ciprofloxacin **151**, and via the triazole linkage shown previously with ciprofloxacin **24** (see 7.4). The compounds were then tested for antibiotic and anti-biofilm activity against *P. aeruginosa*.

#### 8.1.1 Head groups

The head groups used in this study are shown in Figure 37. The cyclohexanol derivatives were synthesised as a diastereomerically pure racemate, whereas the cyclopentanol derivatives were synthesised as separate enantiomers. Although the timescale of this project prevented the inclusion of the cyclopentanone derivatives, these could be included in future work. The 2-methoxybenzene derivatives do not have precedents as quorum sensing modulators in the literature, but they were included so as to be compared with the 3-methoxybenzene derivatives.

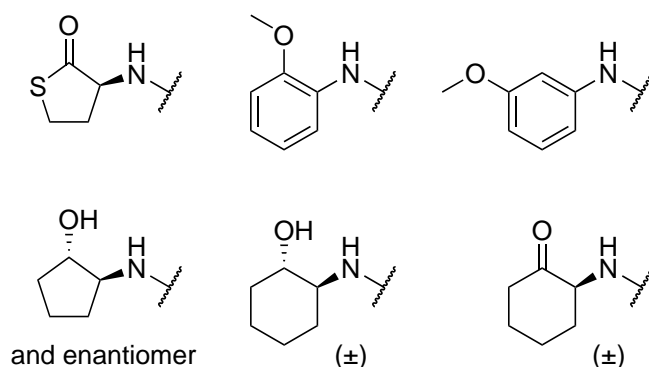
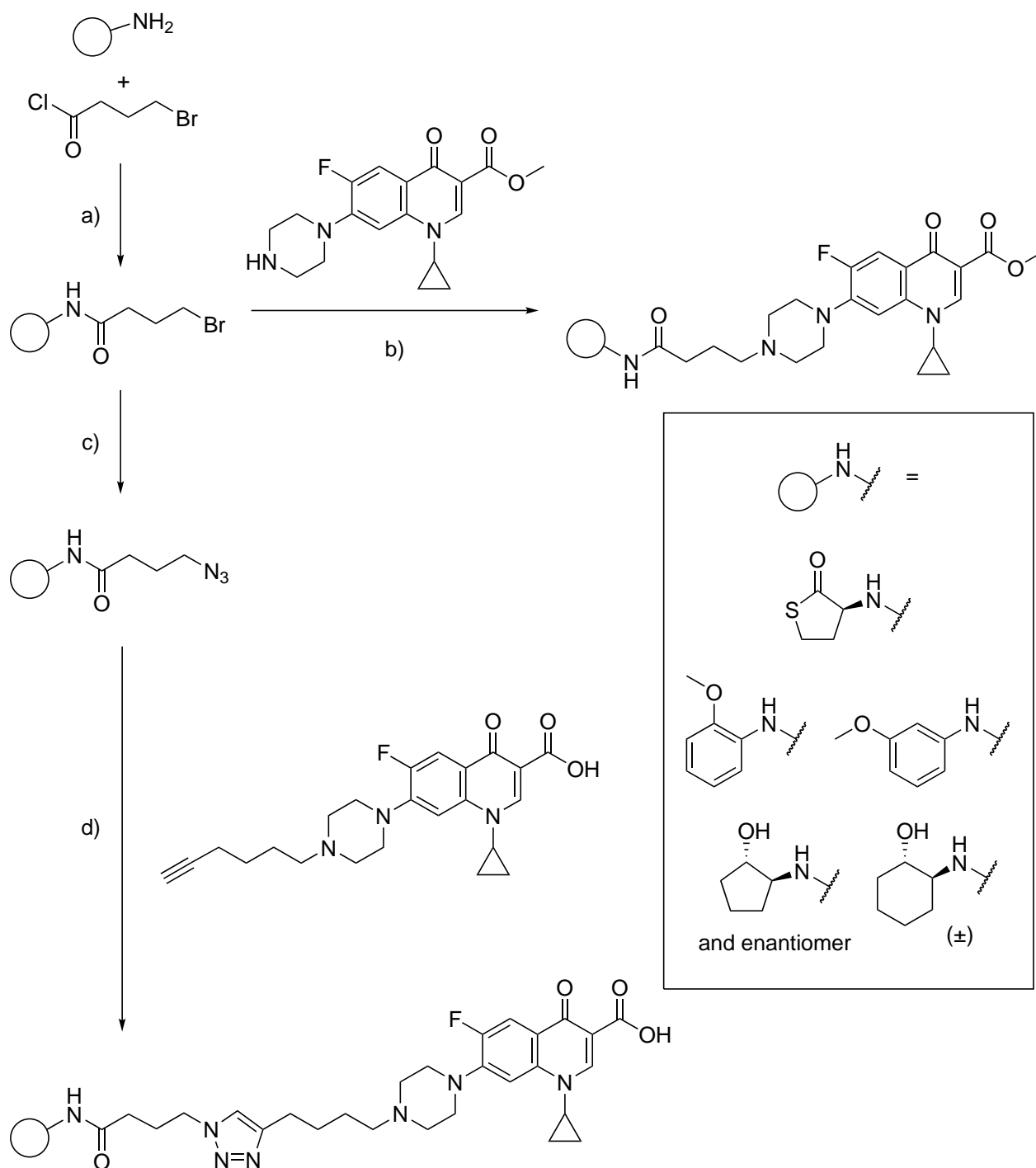


Figure 37: The head groups used in this section.

#### 8.1.2 Library construction

As Ganguly *et al.*<sup>61</sup> (see 5.3.8) synthesised their conjugate from Br-C<sub>4</sub>-HCTL, it was envisaged that a branching strategy could be used to produce two sets of conjugates (see Scheme 19). The first set would be formed by the  $S_N2$  reaction of the relevant bromide with methyl ciprofloxacin **151**. The second set would be made by displacing the bromide with azide, then performing a click reaction with the alkynyl ciprofloxacin derivative **68** made previously to form the triazole-linked product. Cyclohexanone conjugates would be formed by oxidation of the alcohol conjugates.



Scheme 19: General scheme showing the proposed branching synthesis of the HSL analogue-Cip and -CipMe conjugates.

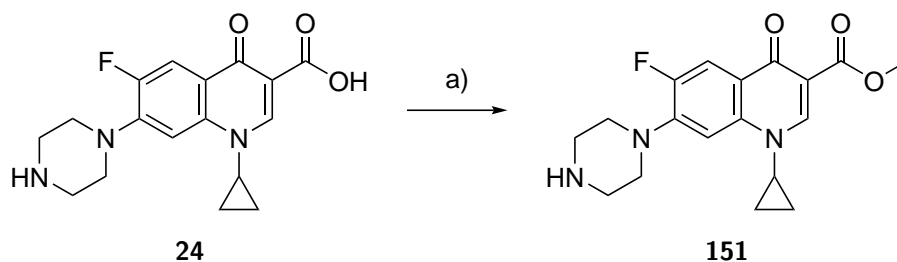
This strategy was successful for most head groups, but multiple side reactions were observed for the amino alcohol head groups and so other routes to these conjugates were investigated (see 8.5).

## 8.2 Synthesis of the homocysteine thiolactone conjugates

### 8.2.1 Synthesis of methyl ciprofloxacin **151**

The synthesis of the analogue conjugates began with the synthesis of methyl ciprofloxacin **151** (CipMe), which would then be attached to the various head groups. Methyl ciprofloxacin **151** was synthesised from ciprofloxacin

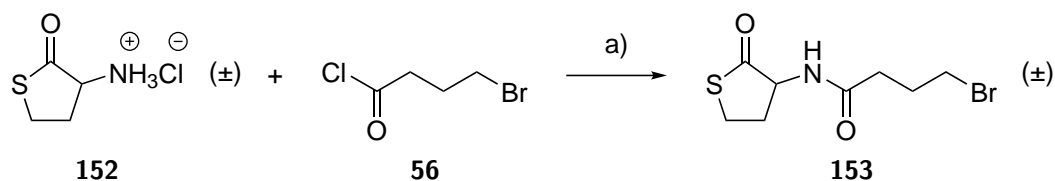
**24** and MeOH in good yield using *para*-toluenesulfonic acid (TsOH) as a catalyst.<sup>216</sup>



Scheme 20: Synthesis of methyl ciprofloxacin **151**. a) TsOH, MeOH, 72 h, reflux, 83%.

### 8.2.2 Synthesis of Br-C<sub>4</sub>-HCTL **153**

The HCTL head group was then attached to the linker to form Br-C<sub>4</sub>-HCTL **153**, in preparation for coupling to methyl ciprofloxacin **151**. Br-C<sub>4</sub>-HCTL **153** was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives **57** and **60**. Br-C<sub>4</sub>-HCTL **153** was isolated in markedly higher yield than that achieved by Ganguly *et al.*<sup>61</sup> (88% vs. 25%). It is possible that this was due to CH<sub>2</sub>Cl<sub>2</sub> being used for the extraction, whereas Ganguly *et al.* used EtOAc.

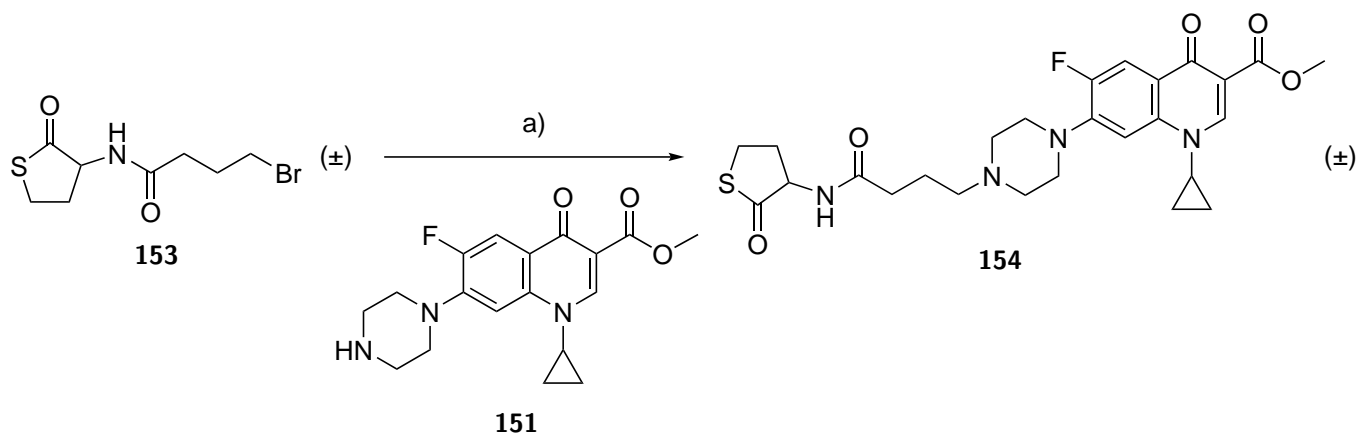


Scheme 21: Synthesis of Br-C<sub>4</sub>-HCTL **153**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, water, 0 °C, 1 h, 88%.

### 8.2.3 Synthesis of the HCTL-CipMe conjugate **154**

The HCTL-CipMe conjugate **154** was synthesised using the procedure outlined by Ganguly *et al.*<sup>61</sup> (see Scheme 22). Monitoring by LCMS showed slow conversion to the product. Br-C<sub>4</sub>-HCTL **153** was presumably consumed by side reactions as 4 eq. were required to reach full conversion. A likely potential side reaction is internal cyclisation of the bromide with the amide NH, and the mass of this molecule was observed by LCMS in the reaction mixture.

Ganguly *et al.* do not quote a yield for this reaction,<sup>61,149</sup> but it is hoped that the 12% achieved here could be improved upon. The side reactions led to the production of an unidentified brown, viscous contaminant which made purification by flash column chromatography (as was used by Ganguly *et al.*) challenging. Preparatory HPLC on a partially purified sample gave enough pure HCTL-CipMe conjugate **154** for biological testing. Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in 8.5.5, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.

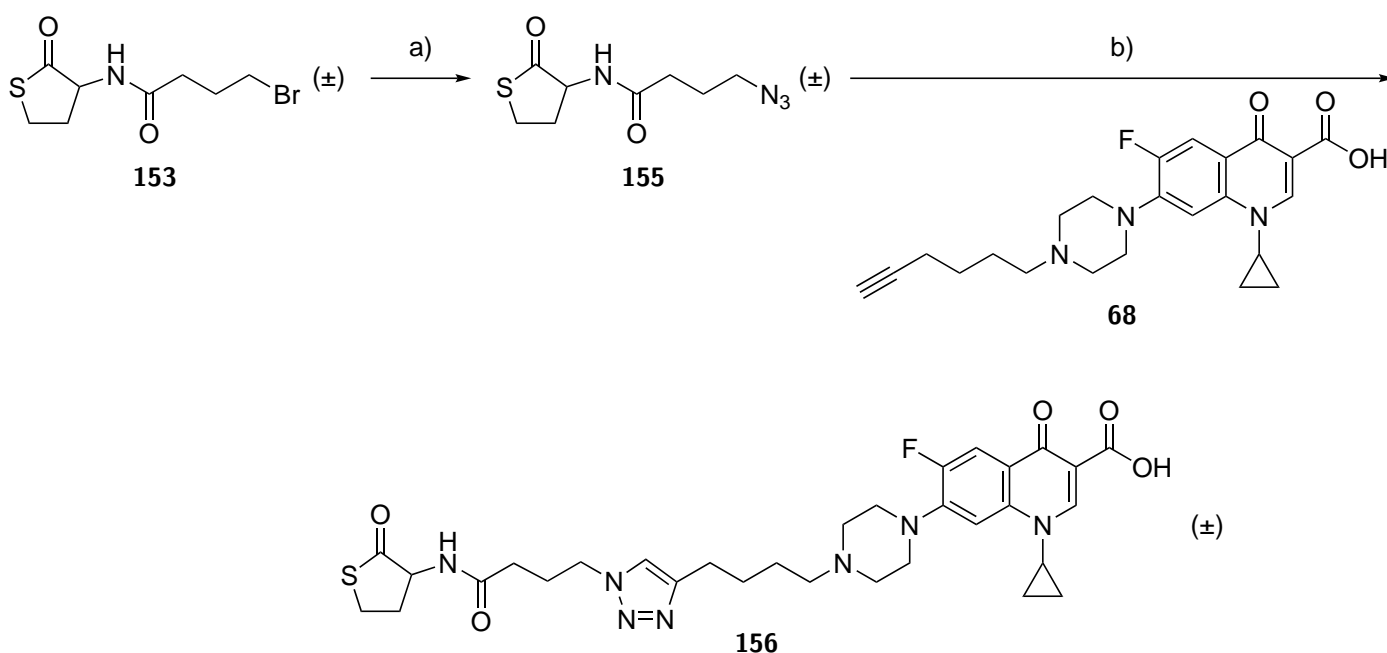


Scheme 22: Synthesis of the HCTL-CipMe conjugate **154**. a)  $\text{K}_2\text{CO}_3$ , acetonitrile, reflux, 24 h, 12%.

#### 8.2.4 Synthesis of the HCTL-Cip triazole conjugate **156**

$\text{Br-C}_4\text{-HCTL}$  **153** was converted into  $\text{N}_3\text{-C}_4\text{-HCTL}$  **155** (see Scheme 22), by an  $\text{S}_{\text{N}}2$  reaction with sodium azide which proceeded in excellent yield.

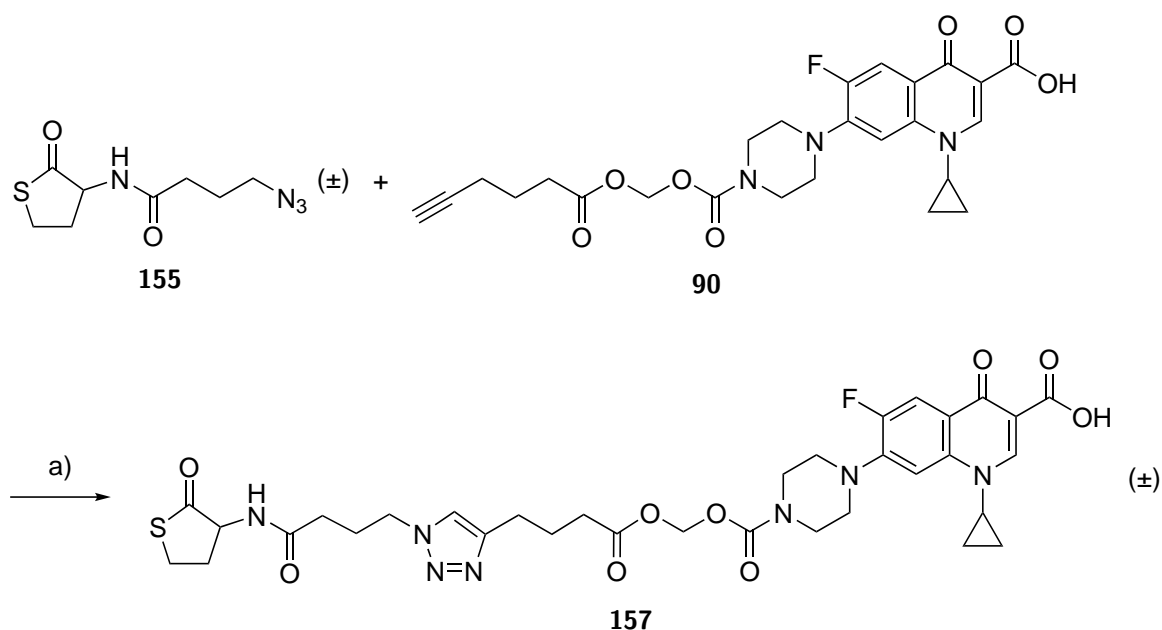
$\text{N}_3\text{-C}_4\text{-HCTL}$  **155** was then subjected to the click reaction conditions optimised previously (see 9.25). The reaction proceeded very slowly at first, as the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. Nonetheless, the HCTL-Cip triazole conjugate **156** was isolated in good yield (see Scheme 23).



Scheme 23: Synthesis of the HCTL-Cip triazole conjugate **156**. a)  $\text{NaN}_3$ , acetonitrile, reflux, 1.5 h, 89%. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate, water, *t*-BuOH, DMSO, r.t., 7 d, 71%.

#### 8.2.5 Synthesis of the cleavable HCTL-Cip triazole conjugate **157**

A cleavable conjugate **157** (see Scheme 24) was also synthesised from  $\text{N}_3\text{-C}_4\text{-HCTL}$  **155** by reaction with a cleavable alkyne-Cip derivative **90** synthesised previously by Professor Eddy Sotelo (see 7.4.3).



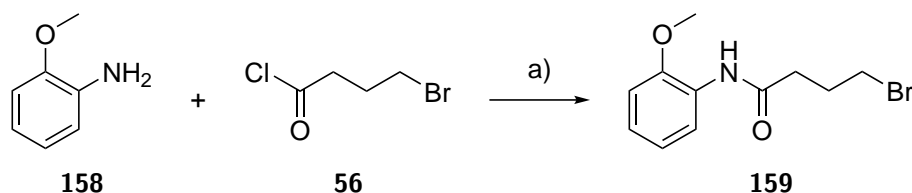
Scheme 24: Synthesis of the cleavable HCTL-Cip triazole conjugate **157**. a) CuI, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 3 h, 5%.

### 8.3 Synthesis of the 2-Methoxybenzene conjugates

#### 8.3.1 Synthesis of Br-C<sub>4</sub>-2-methoxybenzene **159**

Br-C<sub>4</sub>-2-methoxybenzene **159** was synthesised from 2-methoxyaniline **158** and 4-bromobutyryl chloride **56** using Schotten-Baumann conditions in 50% yield (see Scheme 25). Br-C<sub>4</sub>-2-methoxybenzene **159**, like all other 2- and 3-methoxyaniline derivatives mentioned below, appears to be air and/or light sensitive. For example, Br-C<sub>4</sub>-2-methoxybenzene **159** turns from an initially colourless liquid to blue then black if left out on the bench. It is possible that this sensitivity is due to oxidative polymerisation of the aniline,<sup>217,218</sup> but given the lack of catalysis it is likely that small amounts of highly-coloured polymer are being formed.

It is likely that the mediocre yield of Br-C<sub>4</sub>-2-methoxybenzene **159** is caused by degradation during columning, probably due to S<sub>N</sub>2 reactions at the bromide, especially internal cyclisation with the amide NH. It is therefore suggested that in future the compound should be used in its crude form to minimise losses, as it was fairly pure by <sup>1</sup>H NMR before columning.



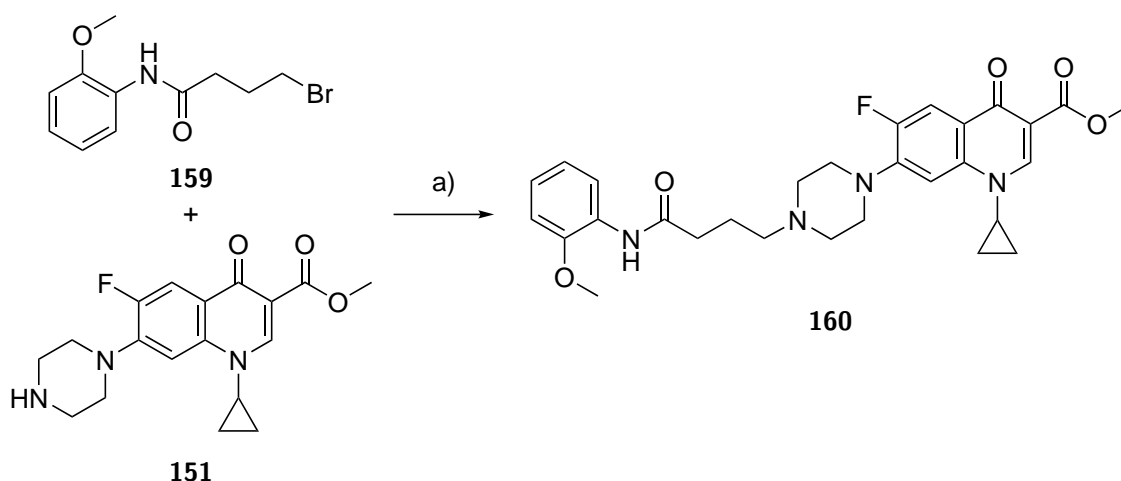
Scheme 25: Synthesis of Br-C<sub>4</sub>-2-methoxybenzene **159**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, water, 0 °C, 1 h, 50%.

#### 8.3.2 Synthesis of the 2-methoxybenzene-CipMe conjugate **160**

The procedure outlined by Ganguly *et al.*<sup>61</sup> was initially attempted in order to synthesise the 2-methoxybenzene-CipMe conjugate **160**, but the reaction was very slow and did not go to completion, presumably due to degradation of Br-C<sub>4</sub>-2-methoxybenzene **159**. New conditions, employing a microwave reactor and 2 eq. of Br-C<sub>4</sub>-2-



methoxybenzene **159** were then attempted, with a much greater conversion observed by LCMS after 4 h (see Scheme 26). However, a poor yield was obtained, possibly due to losses during column chromatography.



Scheme 26: Synthesis of the 2-methoxybenzene-CipMe conjugate **160**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 10%.

### 8.3.3 Synthesis of the 2-methoxybenzene-Cip triazole conjugate **162**

N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene **161** was synthesised from Br-C<sub>4</sub>-2-methoxybenzene **159** by an S<sub>N</sub>2 reaction with sodium azide (see Scheme 27). The yield of N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene **161** (27%) was a lot lower than for N<sub>3</sub>-C<sub>4</sub>-HCTL **155** (89%). However, in this case it may not be better to use the product crude as several impurities were formed during the reaction and could be observed by LCMS (see Figure 38).

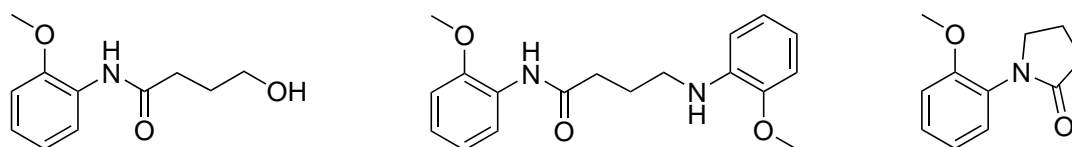
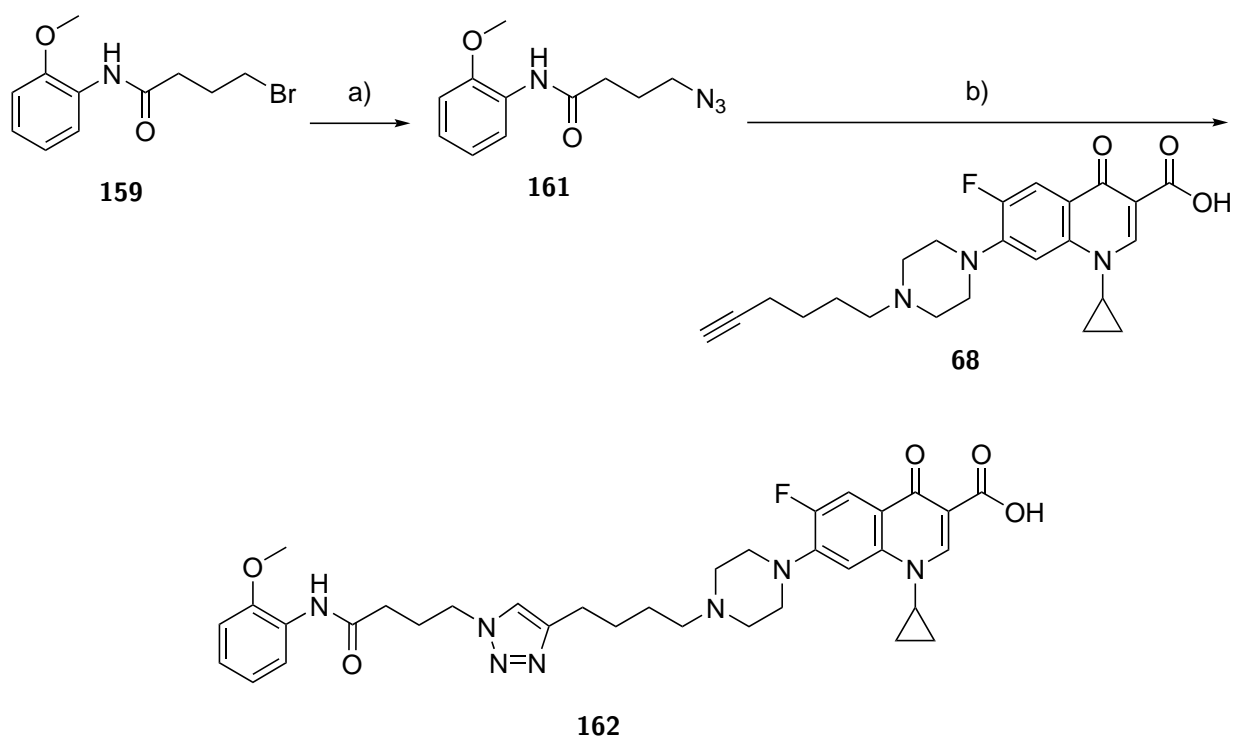


Figure 38: Suspected impurities observed by LCMS during the synthesis of N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene **161**.

The 2-methoxybenzene-Cip triazole conjugate **162** was synthesised using the standard click conditions (see 9.25), with the addition of CH<sub>2</sub>Cl<sub>2</sub> as a co-solvent to aid the dissolution of N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene **161** (see Scheme 27).

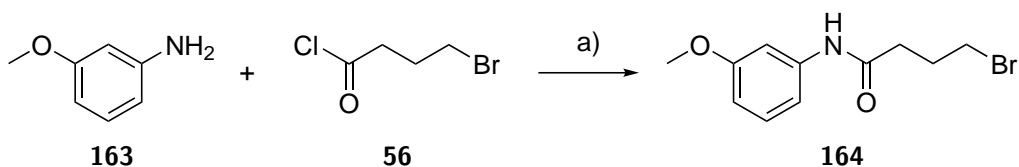


Scheme 27: Synthesis of the 2-methoxybenzene-Cip triazole conjugate **162**. a)  $\text{NaN}_3$ , acetonitrile, reflux, 2 h, 27%. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate, water, *t*-BuOH,  $\text{CH}_2\text{Cl}_2$ , r.t., 16 h, 39%.

## 8.4 Synthesis of the 3-Methoxybenzene conjugates

### 8.4.1 Synthesis of Br- $\text{C}_4$ -3-methoxybenzene **164**

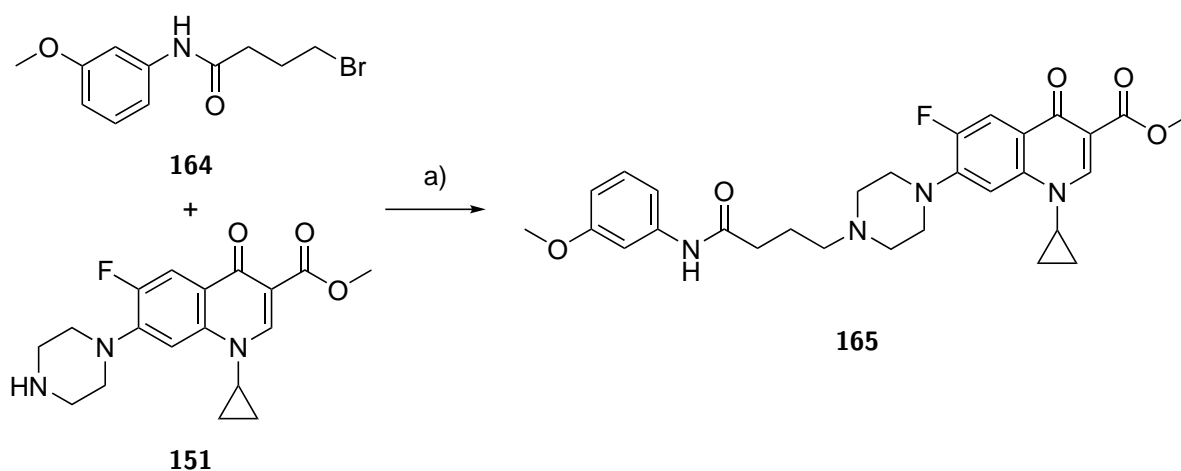
Br- $\text{C}_4$ -3-methoxybenzene **164** was synthesised from 3-methoxyaniline **163** and 4-bromobutyryl chloride **56** using Schotten-Baumann conditions as above in almost identical (50%) yield (see Scheme 28).



Scheme 28: Synthesis of Br- $\text{C}_4$ -3-methoxybenzene **159**. a)  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ , water,  $0^\circ\text{C}$ , 1 h, 50%.

### 8.4.2 Synthesis of the 3-methoxybenzene-CipMe conjugate **165**

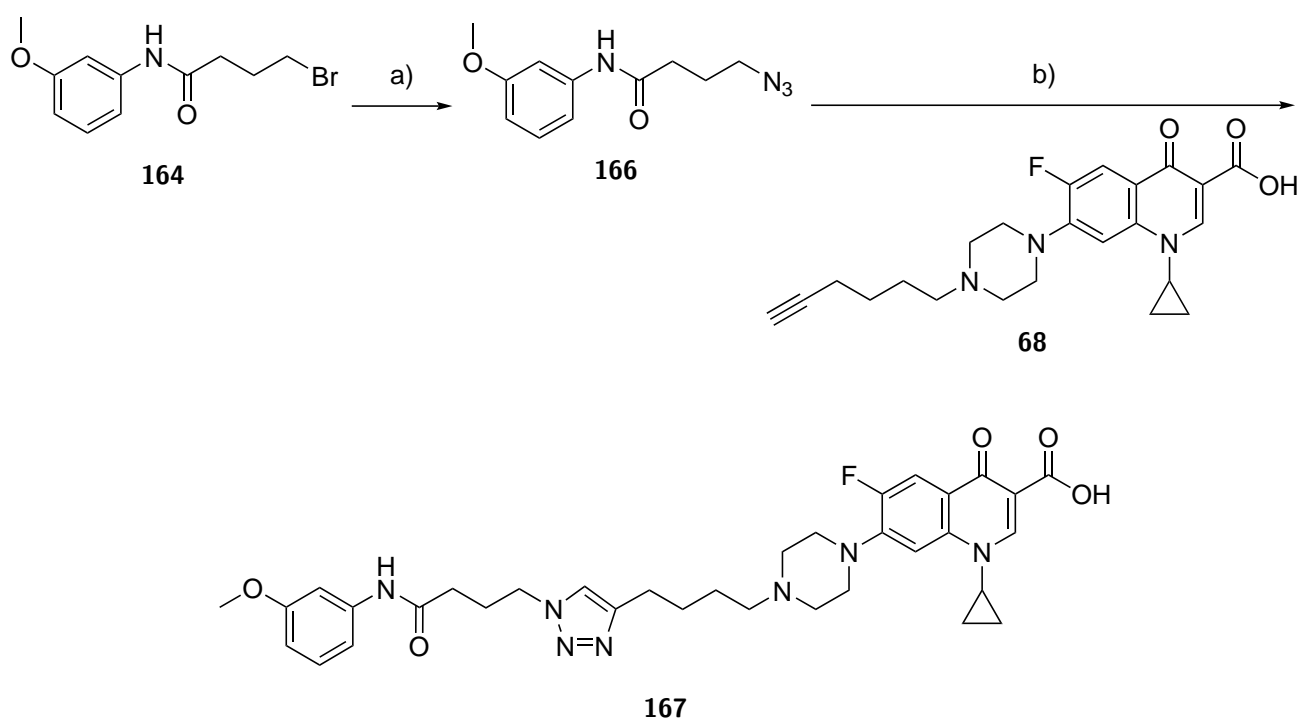
The 3-methoxybenzene-CipMe conjugate **165**, was synthesised as above, in similar yield (see Scheme 29).



Scheme 29: Synthesis of the 3-methoxybenzene-CipMe conjugate **165**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 11%.

#### 8.4.3 Synthesis of the 3-methoxybenzene-Cip triazole conjugate **167**

N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene **161** and the 3-methoxybenzene-Cip triazole conjugate **167** were synthesised as above, in similar yields (see Scheme 29 and Scheme 30).

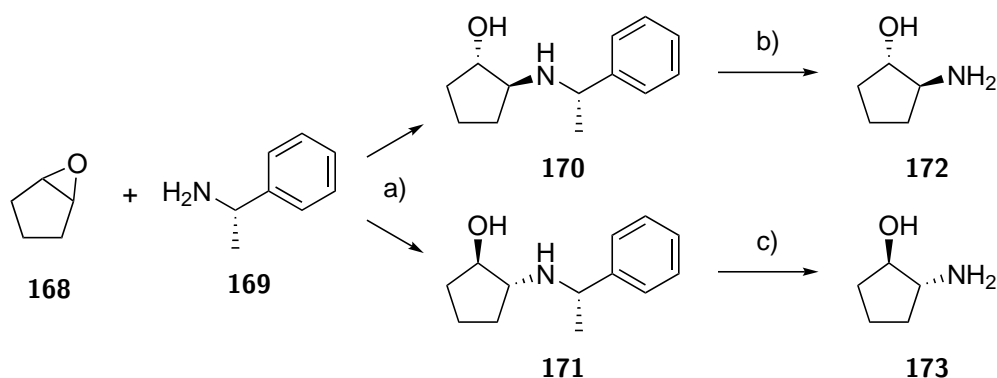


Scheme 30: Synthesis of the 3-methoxybenzene-Cip triazole conjugate **167**. a) NaN<sub>3</sub>, acetonitrile, reflux, 7 h, 17%. b) CuSO<sub>4</sub>, THPTA, sodium ascorbate, water, *t*-BuOH, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 2 h, 5%.

## 8.5 Synthesis of the cyclopentanol conjugates

### 8.5.1 Synthesis of the 2-aminocyclopentan-1-ol head groups **172** and **173**

Synthesis of the cyclopentanol derivatives began with the synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** and (1*R*,2*R*)-2-aminocyclopentan-1-ol **173** (see Scheme 31), using a procedure reported by Overman and Sugai.<sup>219–221</sup> These precursors were synthesised by opening cyclopentene oxide **168** using (*S*)-1-phenylethan-1-amine **169** to give approximately equal amounts of two diastereomers, **170** and **171**, which were separated using column chromatography. The removal of the methylbenzyl groups proved more difficult than expected, with the conditions reported by Overman and Sugai<sup>220</sup> yielding only a salt of the starting material. After several attempts under various conditions (including using the free amine vs. the salt, varying the temperature, ensuring the dryness of the reagents and adding acetic acid), an approach using H<sub>2</sub> gas was attempted (see Table 9). This proceeded smoothly at 5 atm to give the two enantiomers of 2-aminocyclopentan-1-ol, **172** and **173**, both in quantitative yield.



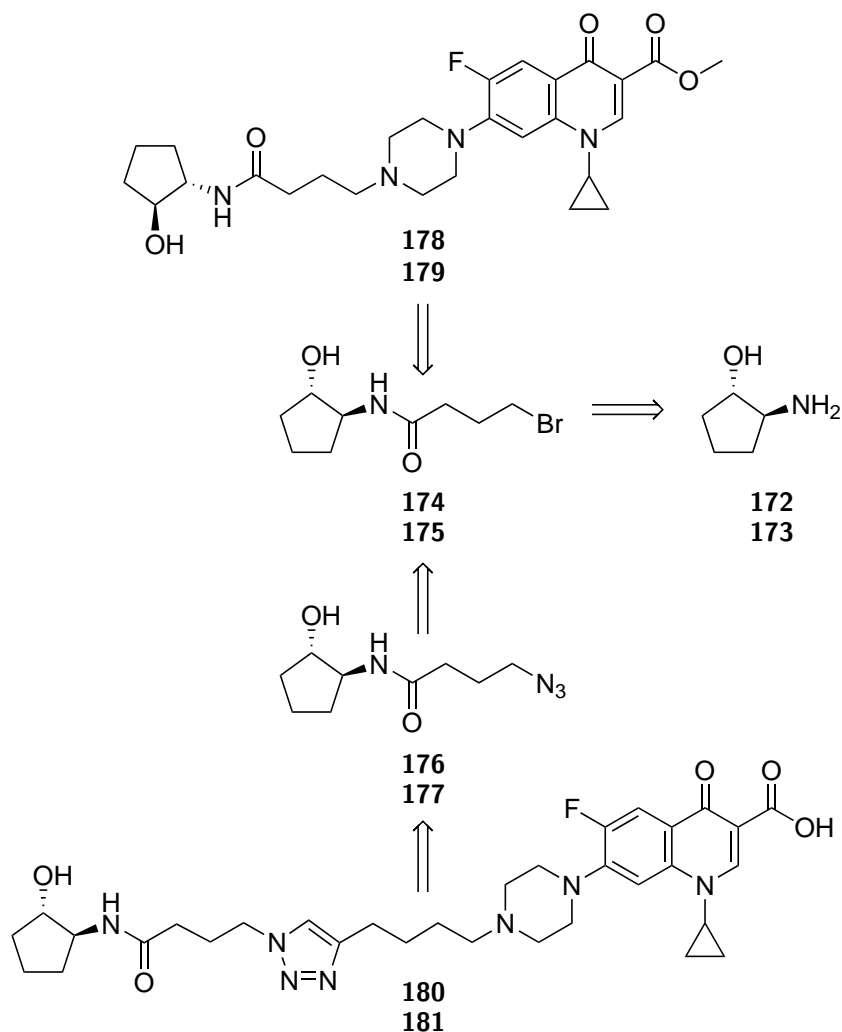
Scheme 31: Synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** and (1*R*,2*R*)-2-aminocyclopentan-1-ol **173**. a) AlMe<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, **170** (*SSS*): 35%, **171** (*RRS*): 32%. b) See Table 9. c) Pd(OH)<sub>2</sub>, MeOH, H<sub>2</sub>, 5 atm, r.t., 1 d, >99%.

Conditions	Temperature and pressure	Time	Result
<b>170</b> · HCl, ammonium formate, 10% Pd/C, DMF	r.t., 1 atm	2 d	<b>170</b> salt
<b>170</b> , ammonium formate, 10% Pd/C, DMF	r.t., 1 atm	2 d	<b>170</b> salt
<b>170</b> · HCl, ammonium formate, 10% Pd/C, dry DMF	r.t., 1 atm	2 d	<b>170</b> salt
<b>171</b> , ammonium formate, 10% Pd/C, dry DMF	r.t., 1 atm	2 d	<b>171</b> salt
<b>170</b> , ammonium formate, 10% Pd/C, dry DMF	70 °C, 1 atm	1 d	<b>170</b> salt
<b>170</b> , ammonium formate, 10% Pd/C, dry DMF, AcOH	70 °C, 1 atm	1 d	Complex mixture
<b>170</b> · HCl, dry ammonium formate, 10% Pd/C, dry DMF	120 °C, 1 atm	7 d	Complex mixture
<b>170</b> · HCl, Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 1 atm	1 d	<b>170</b> salt
<b>170</b> · HCl, Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 3.4 atm	1 d	<b>172</b> salt, <b>170</b> salt, and an unidentified compound (approx. 7:2:10 by <sup>1</sup> H NMR)
<b>170</b> , Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 5 atm	1 d	<b>172</b> , >99% yield

Table 9: Conditions attempted for the synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** and (1*R*,2*R*)-2-aminocyclopentan-1-ol **173** (see Scheme 31).

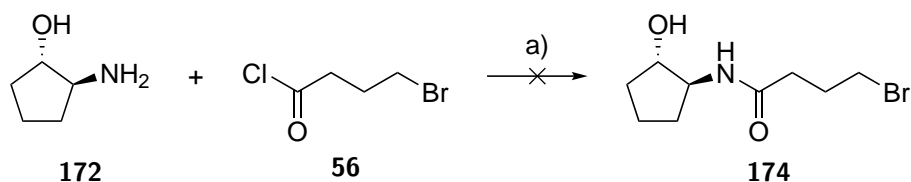
### 8.5.2 Initial branching route

An initial retrosynthesis of the conjugates is shown in Scheme 32, and follows a similar path to previous conjugates.



Scheme 32: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*), and the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*). *SS* enantiomers are shown, but both are implied.

Synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **174** from (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** and 4-bromobutyryl chloride **56** was attempted using Schotten-Baumann conditions (see Scheme 33). However, a large number of impurities were observed by LCMS (see Figure 39), and so three new strategies were attempted: protection of the alcohol (see 8.5.3), using 4-chlorobutyryl chloride **192** as the linker instead of 4-bromobutyryl chloride **56** (see 8.5.4), and installing the linker on methyl ciprofloxacin **151** and then attaching the head group by peptide coupling (see 8.5.5).



Scheme 33: Attempted synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **174**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, water, 0 °C, 2 h.

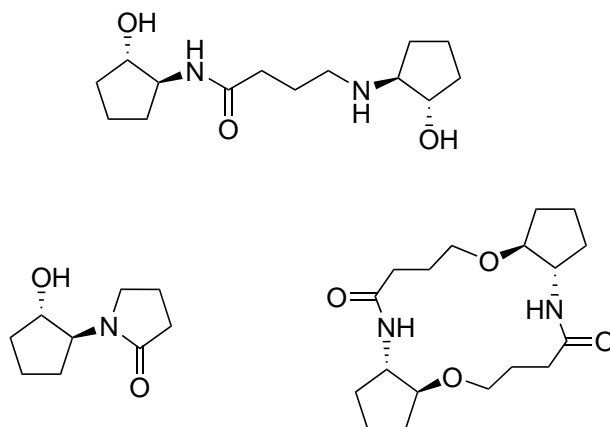
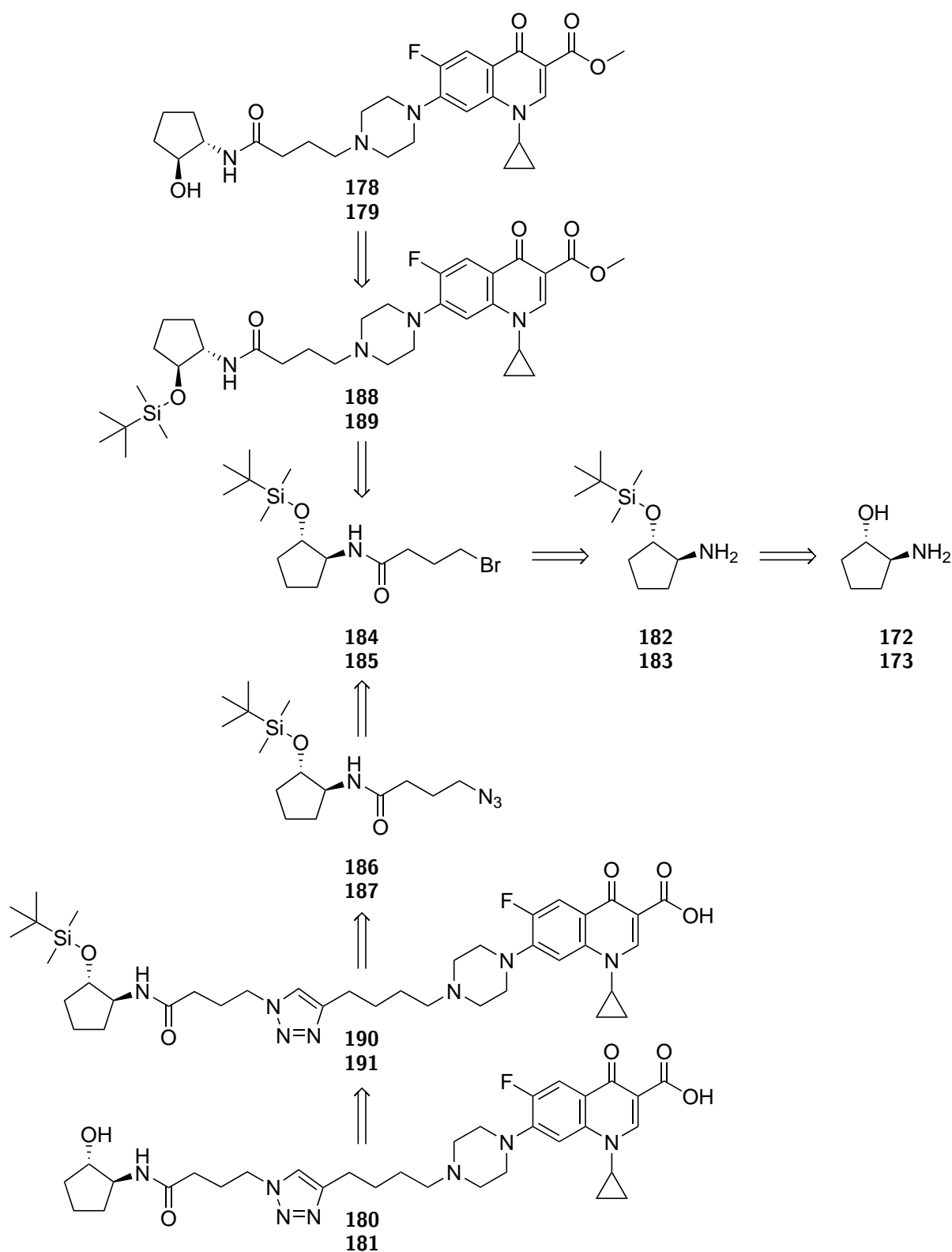


Figure 39: Suspected impurities observed by LCMS during the synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **174**. Regiochemistry is speculative.

### 8.5.3 TBDMS protection route

The first attempt at an alternative strategy for the synthesis of the conjugates involved TBDMS protection of the alcohol (see Scheme 34). It was envisaged that protection would eliminate enough of the side reactions with products shown in Figure 39 that intermediates Br-C<sub>4</sub>-cyclopentanol-(*SS*) **174** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **176** could be purified. The TBDMS group could be removed later in the synthesis using TBAF or acid.



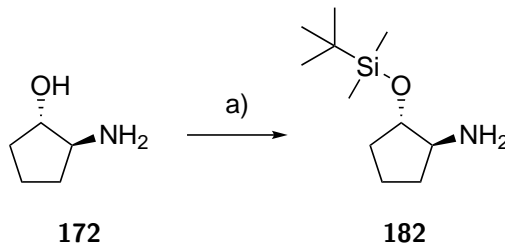
Scheme 34: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*), and the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) using a TBDMS protection strategy. *SS* enantiomers are shown, but both are implied.

#### 8.5.3.1 Synthesis of TBDMS-protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **172**

The synthesis began with the optimisation of the protection of (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** with a TBDMS group on the alcohol (see Scheme 36). This reaction proved more problematic than expected, possibly



due to the amine group interfering with the reaction at the alcohol and/or the high polarity of the starting material causing problems with solubility in the reaction mixture and extraction during the work-up. Conditions attempted are summarised in Table 10. Protection attempts using TBDMSCl were generally unsuccessful, but eventually a method employed by Wu et. al<sup>222</sup> using TBDMSOTf was found to produce the desired product in excellent yield. Water was used for the work-up rather than NH<sub>4</sub>Cl (sat. aq.), as the acidic work-up protonated the product. The TEA was removed during column chromatography instead.



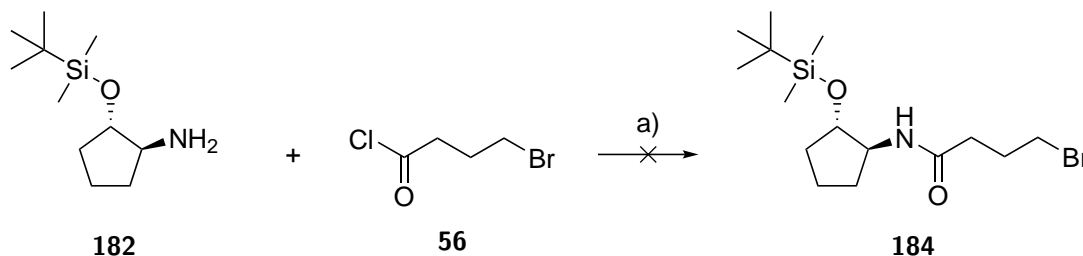
Scheme 35: Synthesis of TBDMS protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **182**. a) See Table 10.

Conditions	Temperature	Time	Result
TBDMSCl, DMAP, TEA, CH <sub>2</sub> Cl <sub>2</sub> <sup>223</sup>	r.t.	18 h	Trace of <b>182</b> , mostly <b>172</b>
TBDMSCl, imidazole, CH <sub>2</sub> Cl <sub>2</sub> <sup>224</sup>	0 °C	1 h	<b>172</b>
TBDMSCl, DBU, acetonitrile <sup>225</sup>	0 °C	1 d	<b>172</b>
TBDMSOTf, TEA, CH <sub>2</sub> Cl <sub>2</sub> , <sup>222</sup> aq. workup then column	0 °C	6 h	<b>182</b> , 98% yield

Table 10: Conditions attempted for the synthesis of (1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentan-1-amine **182** (see Scheme 36).

#### 8.5.3.2 Synthesis of Br-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **184**

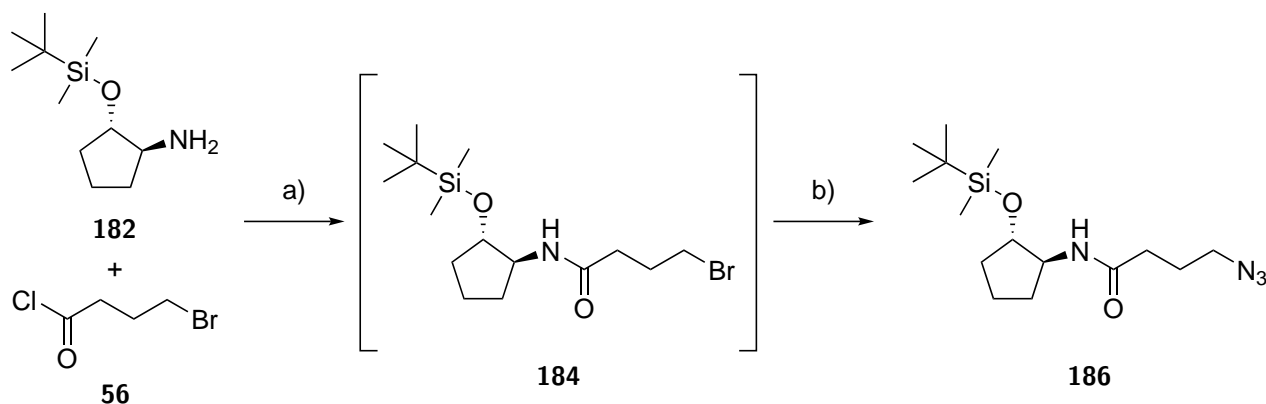
The TBDMS protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **182** was reacted with 4-bromobutyryl chloride **56** to form Br-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **184**. The reaction was observed to go to completion by TLC, but it became apparent that the product was reacting further during concentration and purification. Adding sodium azide to the mixture obtained after the failed purification attempts was observed to convert the remaining Br-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **184** to N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186**. A sequential one-pot reaction was therefore used, so that the reactive intermediate did not need to be isolated.



Scheme 36: Attempted synthesis of Br-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **184**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, water, 0 °C, 2 h.

#### 8.5.3.3 Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186** by one-pot reaction

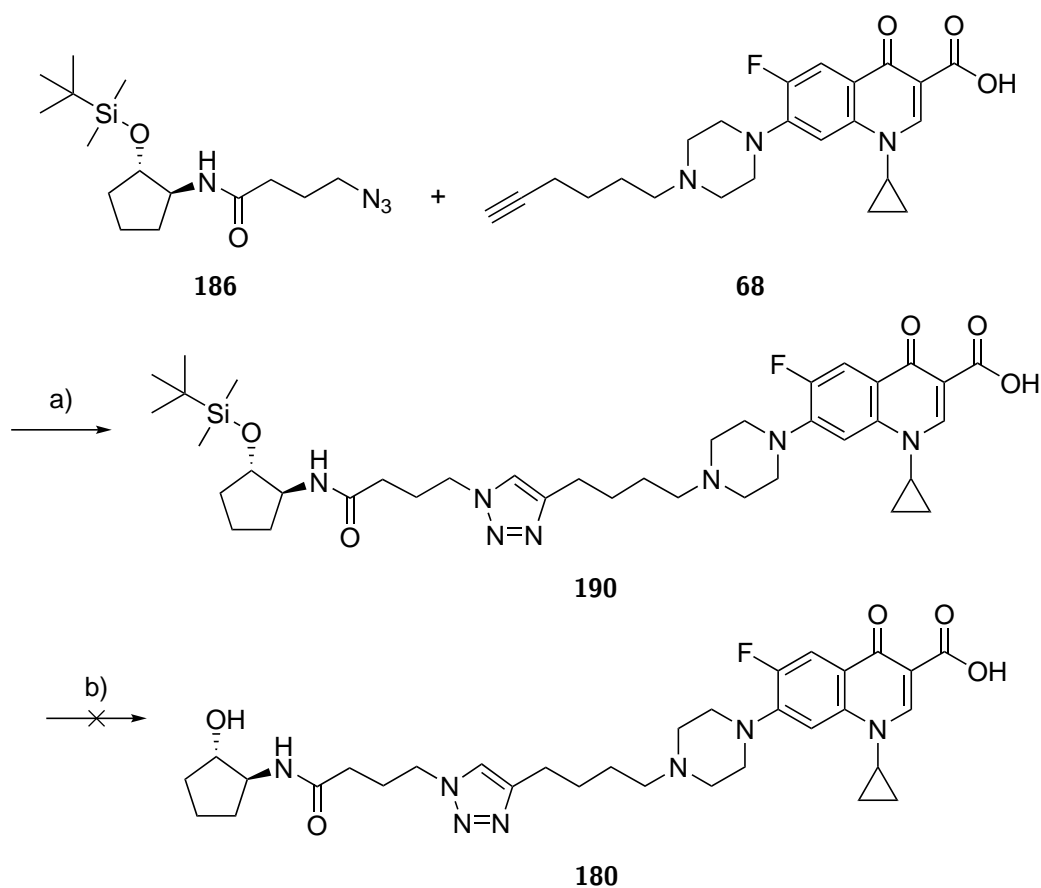
N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186** was finally synthesised by a two-step, one-pot reaction. Schotten-Baumann conditions were used to form the bromide. The water was then removed, and DMF and sodium azide were added. N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186** was produced in excellent yield.



Scheme 37: Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, water, 0 °C, 3 h. b) NaN<sub>3</sub>, DMF, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 3 h. 99% over 2 steps.

#### 8.5.3.4 Synthesis of the (*SS*)-TBDMS-cyclopentanol-Cip triazole conjugate **190**

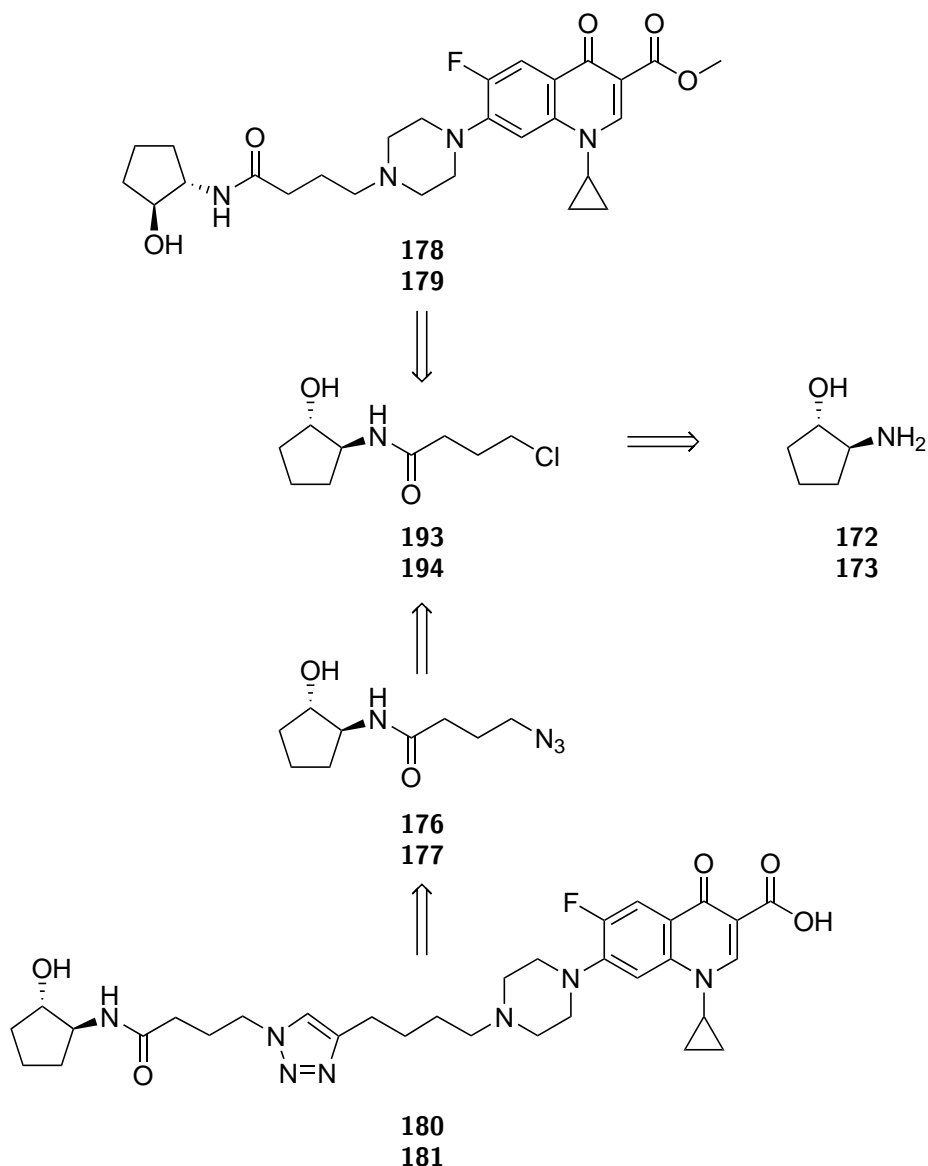
N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **186** and the alkynyl ciprofloxacin derivative **68** were subjected to standard click conditions (see 9.25), and the (*SS*)-TBDMS-cyclopentanol-Cip triazole conjugate **190** was synthesised in very good yield. However, removal of the TBDMS group proved difficult. Deprotection using 1.5 eq. TBAF in THF proceeded slowly, reaching completion in 5 d. Increasing the amount of TBAF to 8 eq. allowed the reaction to proceed overnight. Purification of the final conjugate **180** by column chromatography was not successful due to streaking and poor separation. Purification using DOWEX resin and CaCO<sub>3</sub><sup>226</sup> was attempted, but the product could not be recovered from the resin. The purification method could probably be optimised, e.g. by varying the solvent used with the resin, but ultimately this route was abandoned due to the reduction in number of steps afforded by the two methods described below.



Scheme 38: Synthesis of the (*SS*)-TBDMS-cyclopentanol-Cip triazole conjugate **190**. a) CuSO<sub>4</sub>, sodium ascorbate, THPTA, water, *t*-BuOH, r.t., 87%. b) TBAF, THF, r.t., 16 h.

#### 8.5.4 Synthesis of the cyclopentanol-Cip triazole conjugates **180** and **181** via chloride intermediates

Given that the side product formation seen in the previous sections was most likely due to S<sub>N</sub>2 attack on the bromide, we decided to use a chloride rather than a bromide intermediate (see Scheme 32 and Scheme 39 to compare). The bromide intermediate was initially chosen as it was used by Ganguly et. al,<sup>61</sup> but it was anticipated that using a chloride would reduce the side reactions seen with the more reactive bromide.



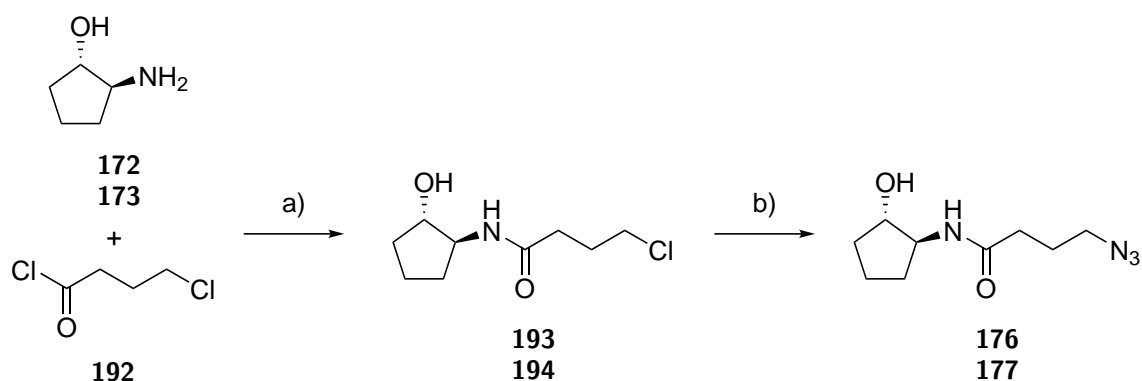
Scheme 39: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*), and the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) via Cl-C<sub>4</sub>-cyclopentanol intermediates **193** (*SS*) and **194** (*RR*). *SS* enantiomers are shown, but both are implied.

Attempts at this route began with the synthesis of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **194**. Standard Schotten-Baumann conditions failed to produce significant amounts of product. If prolonged reaction times were allowed, degradation of the acid chloride to the carboxylic acid was observed. The reason for this is unclear, but it is possible that bromide ions present in small amounts in previous reactions were helping to catalyse the reaction of the acid chloride. Archer *et al.*<sup>227</sup> propose that bromide ions can react with acid chlorides to form acid bromides, which are then more susceptible to nucleophilic attack. As no bromide ions are present in this reaction, different conditions were sought in order to increase the rate.

As (1*R*,2*R*)-2-aminocyclopentan-1-ol **173** is fairly polar, it is likely that it was staying in the aqueous layer to some extent even when deprotonated, thus keeping the two reactants apart. Therefore, the solvent system and base were changed to neat CH<sub>2</sub>Cl<sub>2</sub> and TEA. This produced Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **194** in good yield (64%). Unlike the bromide **174**, the chloride **194** was stable when concentrated.

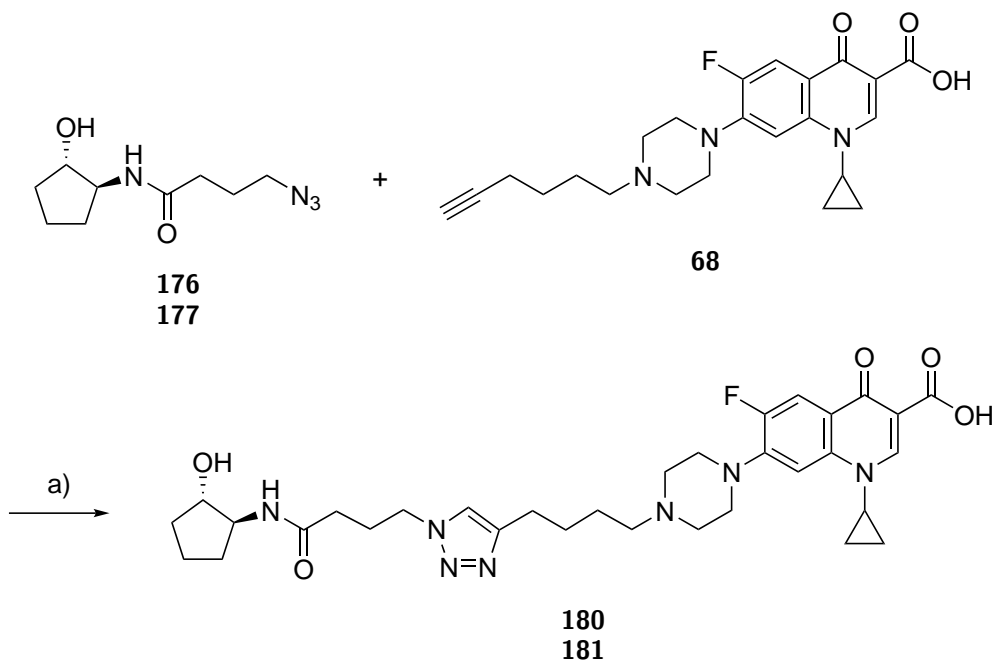
Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **194** was converted to N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) **177** by reaction with sodium azide. The reaction was slower than with previous bromides (~16 h vs. ~2 h), but much cleaner than with Br-C<sub>4</sub>-cyclopentanol-(*SS*) **174** (see 8.5.2).

The enantiomers Cl-C<sub>4</sub>-cyclopentanol-(*SS*) **193** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **176** were synthesised in lower yields, in part because of the smaller amounts being used.



Scheme 40: Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **176** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) **177**. *SS* enantiomers are shown, but both were synthesised. a) TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h, **193** (*SS*): 24%, **194** (*RR*): 64%. b) NaN<sub>3</sub>, acetonitrile, 50 °C, 16 h, **176** (*SS*): 45%, **177** (*RR*): 88%.

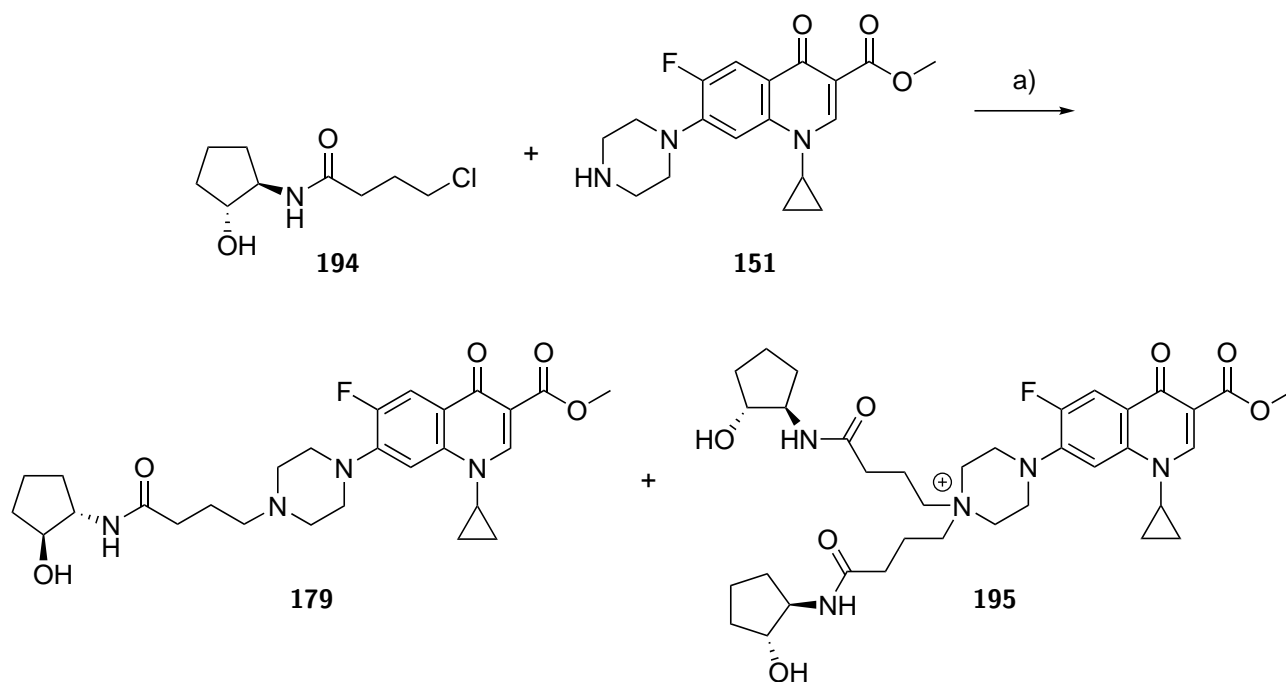
The cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) were successfully synthesised using standard click conditions (see 9.25). Despite low yields (presumably due to problems with purification, including losses on the preparative HPLC column and high polarity leading to losses during extraction from aqueous solvents) enough of the compounds were obtained for biological testing so the purification was not optimised further.



Scheme 41: Synthesis of the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*). *SS* enantiomers are shown, but both were synthesised. a) CuSO<sub>4</sub>, THPTA, sodium ascorbate, water, *t*-BuOH, r.t., 16 h, **180** (*SS*): 22%, **181** (*RR*): 27%.

The S<sub>N</sub>2 reaction of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **194** and methyl ciprofloxacin **151** was attempted (see Scheme 42) using the microwave conditions described previously (see 8.3), to see if the chloride produced better results compared with the bromide. However, as was seen with the other microwave reactions, a substantial amount of the disubstituted product **195** was seen by LCMS (in an approx 1:1 ratio with the desired product

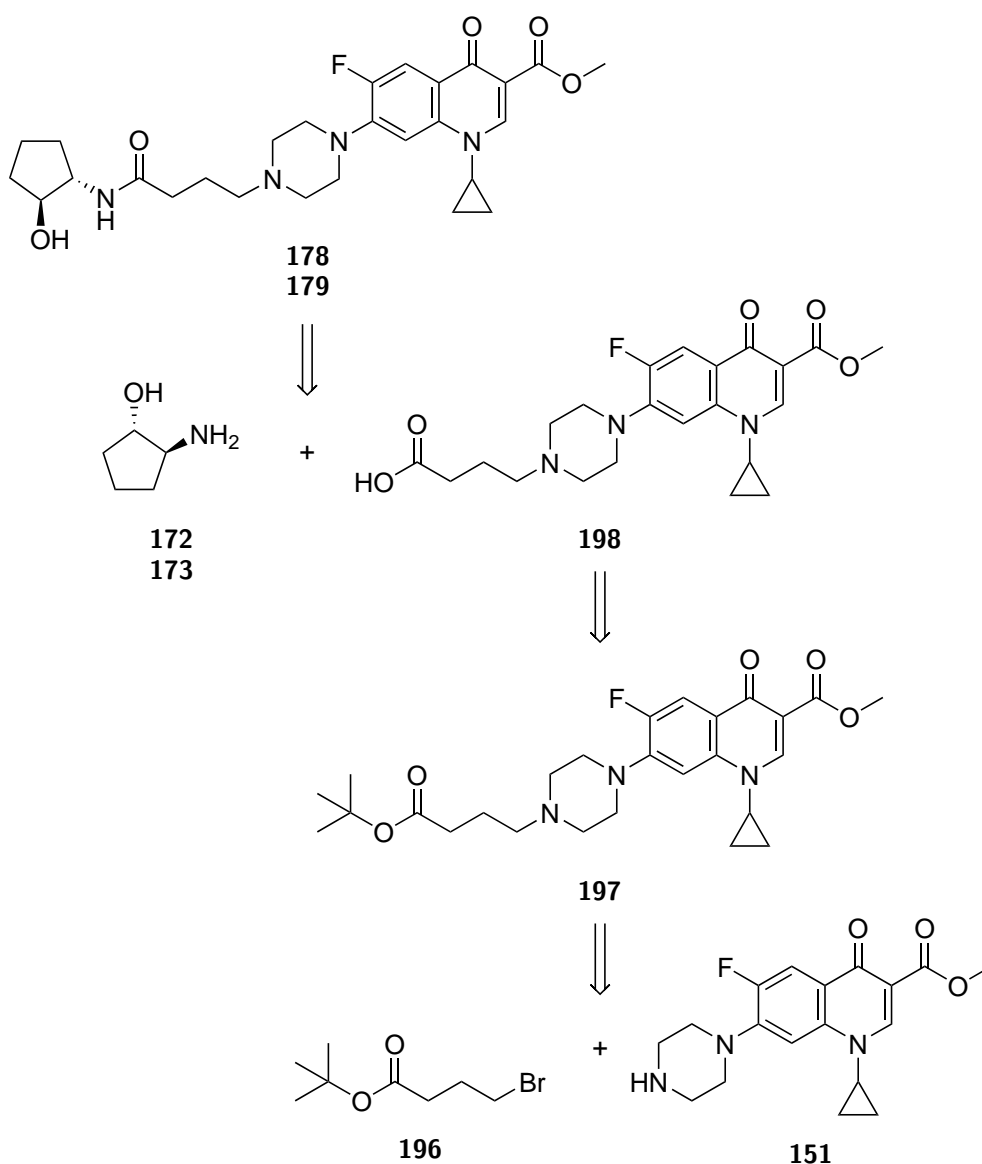
220).



Scheme 42: Attempted synthesis of the cyclopentanol-CipMe-(*RR*) conjugate **179**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C.

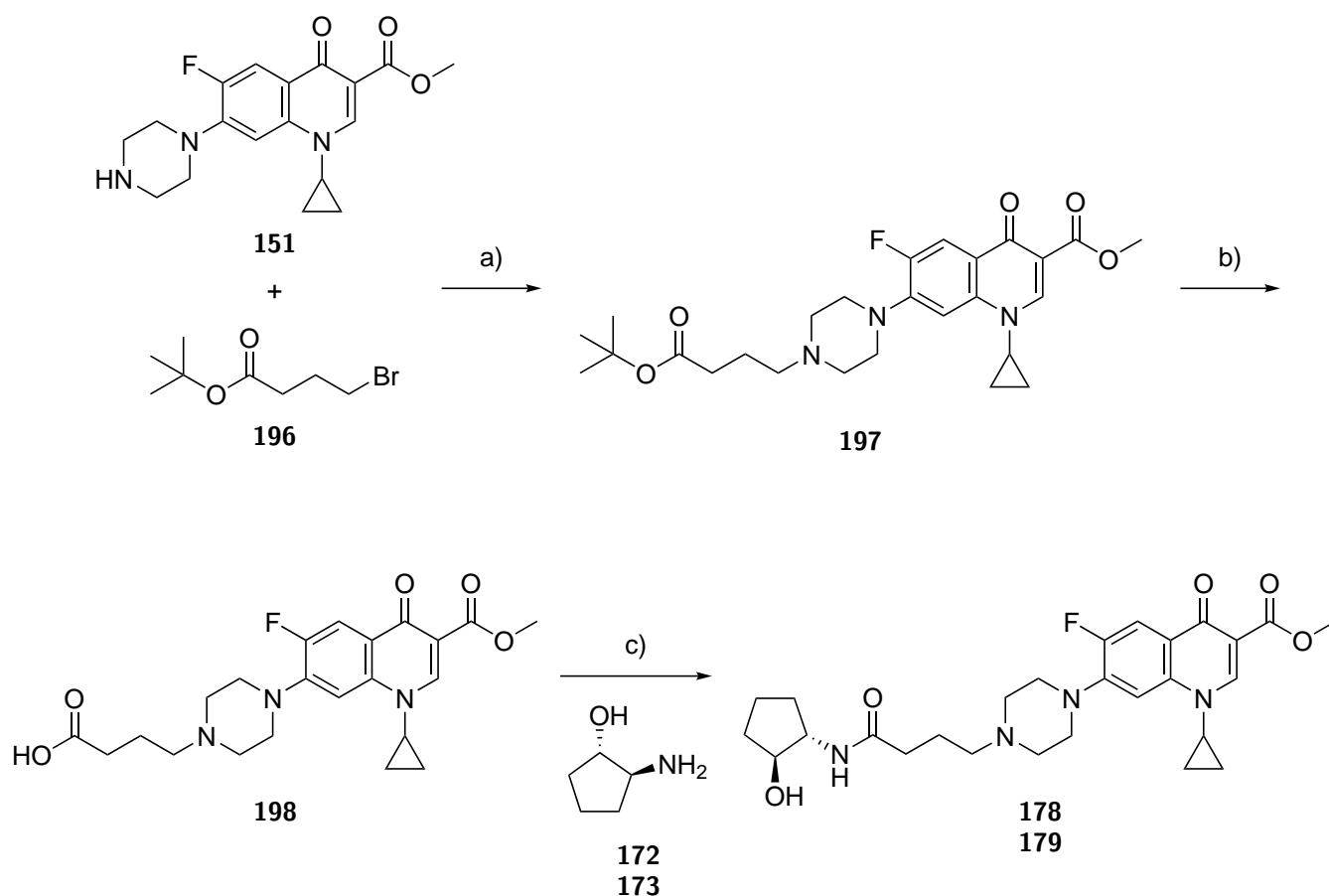
#### 8.5.5 Synthesis of the cyclopentanol-CipMe conjugates **178** and **179** by peptide coupling

Given the side-reactions and low yields associated with the literature synthesis of the  $S_N2$  conjugates proposed by Ganguly et. al,<sup>61</sup> an alternative synthesis was investigated, involving building up the linker on the ciprofloxacin side before coupling with the head group (see Scheme 43).



Scheme 43: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*). *SS* enantiomers are shown, but both are implied.

The first step of the synthesis was an  $S_N2$  reaction between Boc-protected 4-bromobutyric acid **196** methyl ciprofloxacin **151** (see Scheme 44). Intermediate **197** was obtained in acceptable yield after column chromatography (50%). Intermediate **197** was deprotected in excellent yield using TFA in  $\text{CH}_2\text{Cl}_2$  to give carboxylic acid **198**. Scale-up of this reaction allowed the easy synthesis of 600 mg of this useful intermediate, which can be coupled with various amine head-groups to create a library. Carboxylic acid **198** was first coupled with (1*R*,2*R*)-2-aminocyclopentan-1-ol **173** using standard peptide coupling conditions to give cyclopentanol-CipMe conjugate **179**. Purification by column chromatography was attempted twice with poor results, before moving on to using preparative HPLC, which gave **179** cleanly in 39% yield. Coupling was also performed with (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** to give the enantiomer **178** in 55% yield.



Scheme 44: Synthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*) by peptide coupling. *SS* enantiomers are shown, but both were synthesised. a) NaI, TEA, acetonitrile, 100 °C, 16 h, 50%. b) TFA, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, 96%. c) EDC, HOBT, DIPEA, DMF, r.t., 16 h, **178** (*SS*): 55%, **179** (*RR*): 39%.

With (unfortunately not branching) routes to the S<sub>N</sub>2 and click conjugates established (see 8.5.5 and 8.5.4 respectively), attention was turned to the cyclohexanol derivatives.

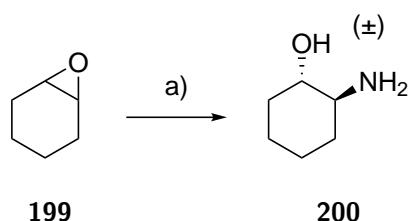
## 8.6 Synthesis of the cyclohexanol conjugates

### 8.6.1 Synthesis of the *trans*-2-aminocyclohexan-1-ol head group **200**

It was decided to produce the cyclohexanol conjugates racemically, with the option of re-synthesising enantiomerically pure versions via the route shown in 8.5.1 if the compounds showed biological activity.

Production of the cyclohexanol conjugates began with the synthesis of *trans*-2-aminocyclohexan-1-ol **200** (see Scheme 45), using a procedure reported by Xue *et al.*<sup>228</sup> Cyclohexene oxide **199** was opened using ammonia in water and methanol. Initially the reaction was carried out at 85 °C in a microwave reactor for 30 min, but a large amount of the disubstituted amine could be seen by LCMS (in a ratio of 4:3 product to impurity by NMR). The reaction was therefore attempted at room temperature, and proceeded overnight in high yield and with minimal side reaction.



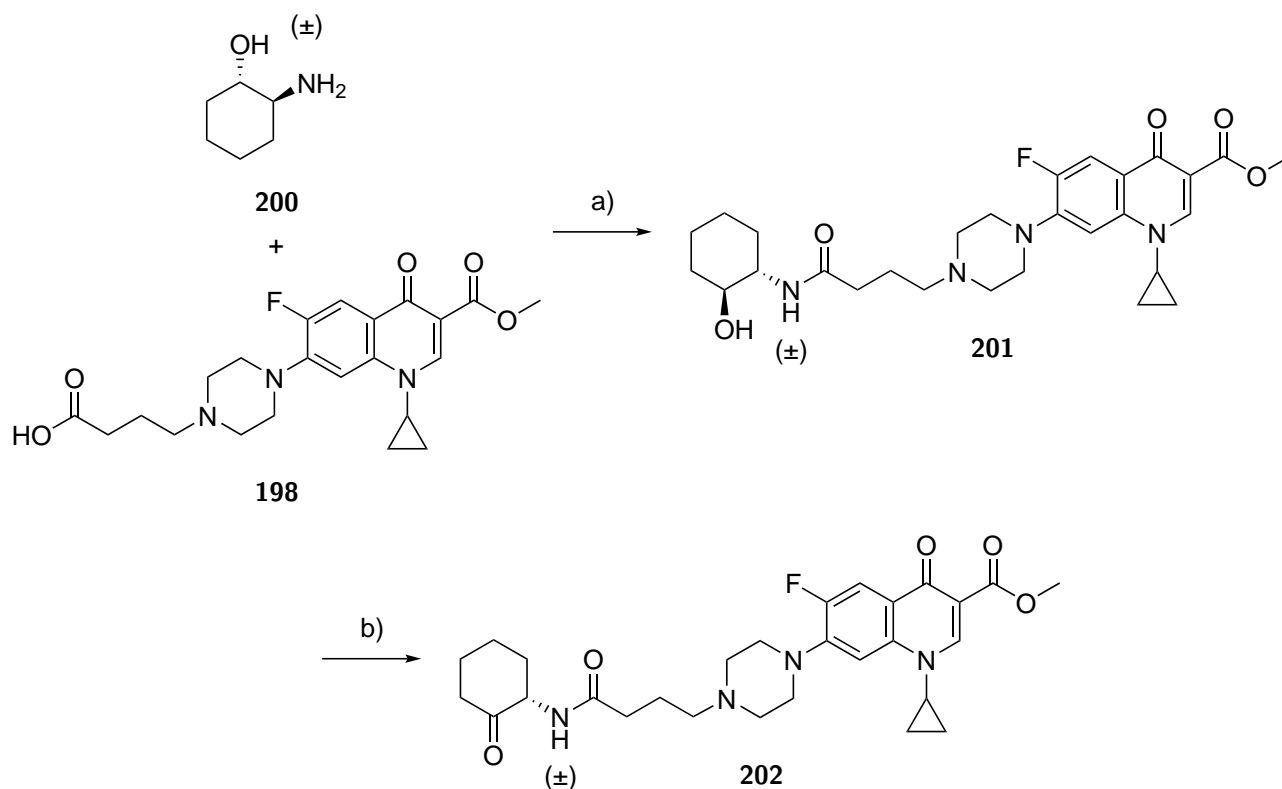


Scheme 45: Synthesis of *trans*-2-aminocyclohexan-1-ol **200**. a)  $\text{NH}_3$ , water, MeOH, r.t., 72 h, 86%.

### 8.6.2 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-CipMe conjugates **201** and **202**

Carboxylic acid **198** was coupled with *trans*-2-aminocyclohexan-1-ol **200** using standard peptide coupling conditions to give *trans*-cyclohexanol-CipMe conjugate **201** in 32% yield.

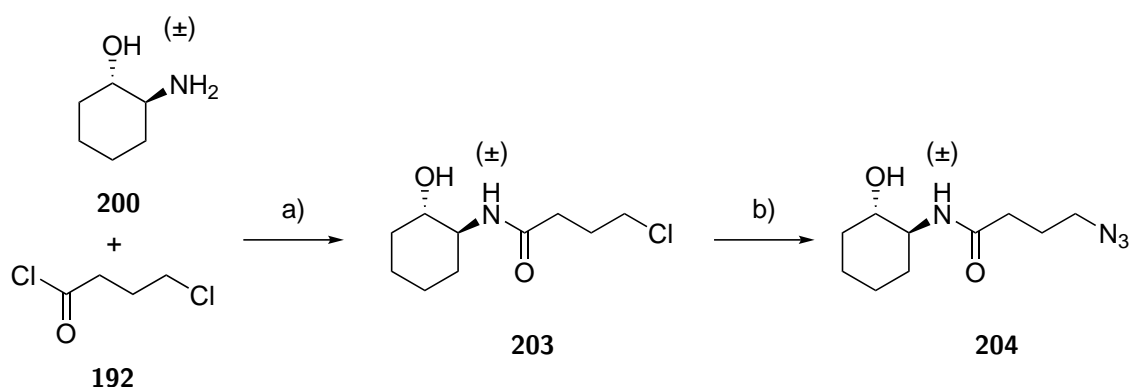
A portion of the *trans*-cyclohexanol-CipMe conjugate **201** was then oxidised to the ketone using Dess-Martin periodinane and the product was isolated in good yield.



Scheme 46: Synthesis of the cyclohexanol-CipMe conjugate **201** and the cyclohexanone-CipMe conjugate **202**. a) EDC, HOBT, DIPEA, DMF, r.t., 16 h, 32%. b) DMP,  $\text{CH}_2\text{Cl}_2$ , r.t., 6 h, 69%.

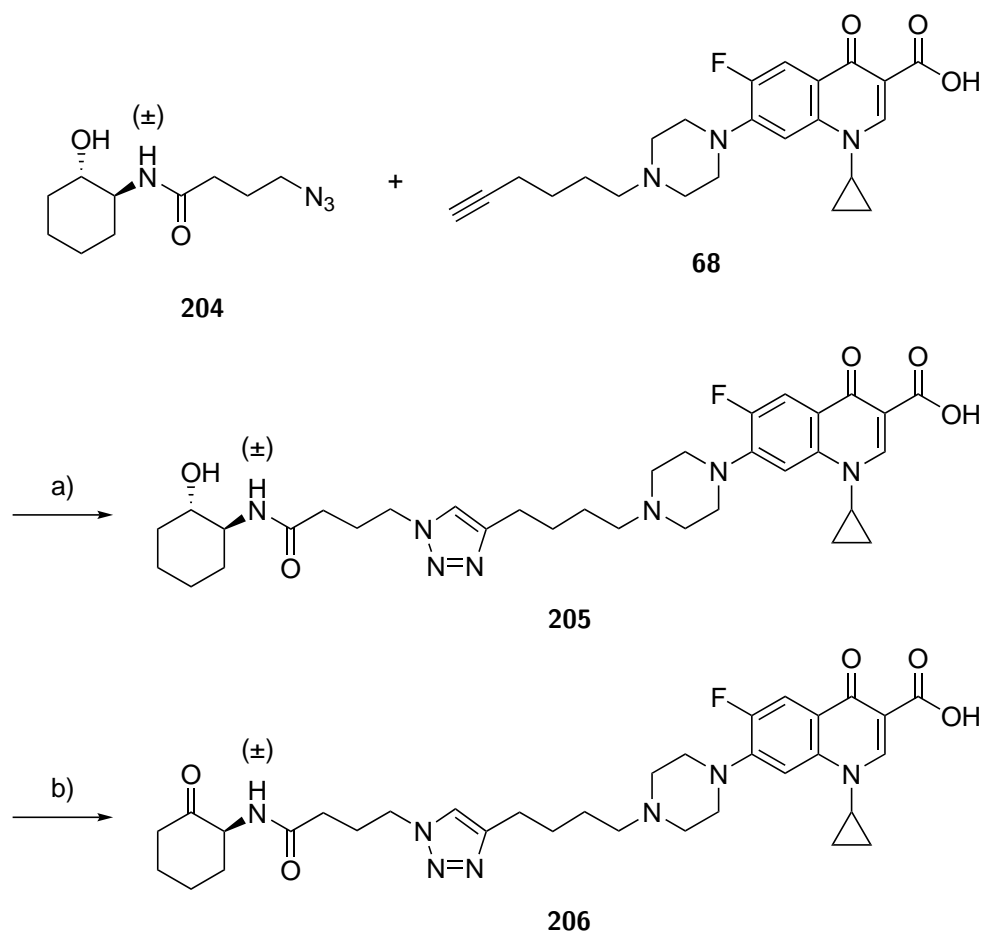
### 8.6.3 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-Cip triazole conjugates **205** and **206**

The triazole conjugates were synthesised using the route described in 8.5.4.  $\text{Cl-C}_4$ -*trans*-cyclohexanol **203** was synthesised in good yield from *trans*-2-aminocyclohexan-1-ol **200** and 4-chlorobutyryl chloride **192**.  $\text{Cl-C}_4$ -*trans*-cyclohexanol **203** was then converted to  $\text{N}_3\text{-C}_4$ -*trans*-cyclohexanol **204** by reaction with sodium azide in excellent yield.



Scheme 47: Synthesis of N<sub>3</sub>-C<sub>4</sub>-*trans*-cyclohexanol **204**. a) TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 30 min, 76%. b) NaN<sub>3</sub>, acetonitrile, 50 °C, 16 h, 98%.

The *trans*-cyclohexanol-Cip triazole conjugate **205** was synthesised using standard click conditions (see 9.25) in 49% yield. A portion of the *trans*-cyclohexanol-Cip triazole conjugate **205** was then oxidised to the ketone using the same conditions used for the cyclohexanone-CipMe conjugate (see 8.6.2) in very good yield.



Scheme 48: Synthesis of the *trans*-cyclohexanol-Cip triazole conjugate **205** and the cyclohexanone-Cip triazole conjugate **206**. a) CuSO<sub>4</sub>, THPTA, sodium ascorbate, water, *t*-BuOH, r.t., 16 h, 49%. b) DMP, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 4 h, 78%.

## 8.7 Biological testing

The biological testing presented in this section was planned by me but carried out by Tom O'Brien, a PhD student in the Department of Biochemistry.

The HSL analogue-Cip(Me) conjugates (see Figure 40), as well as C<sub>4</sub>-HSL **19**, ciprofloxacin **24**, methyl ciprofloxacin **151**, the alkynyl ciprofloxacin derivative **68**, the *tert*-butyl ester methyl ciprofloxacin derivative **197** and the carboxylic acid methyl ciprofloxacin derivative **198** were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* PAO1<sup>180</sup> and YM64.<sup>181</sup> All compounds were tested in triplicate, and the ratio of the standard deviation (SD) to the mean was less than 1 for all data points except one (**68** at 25  $\mu$ l in PAO1 at 8 h).

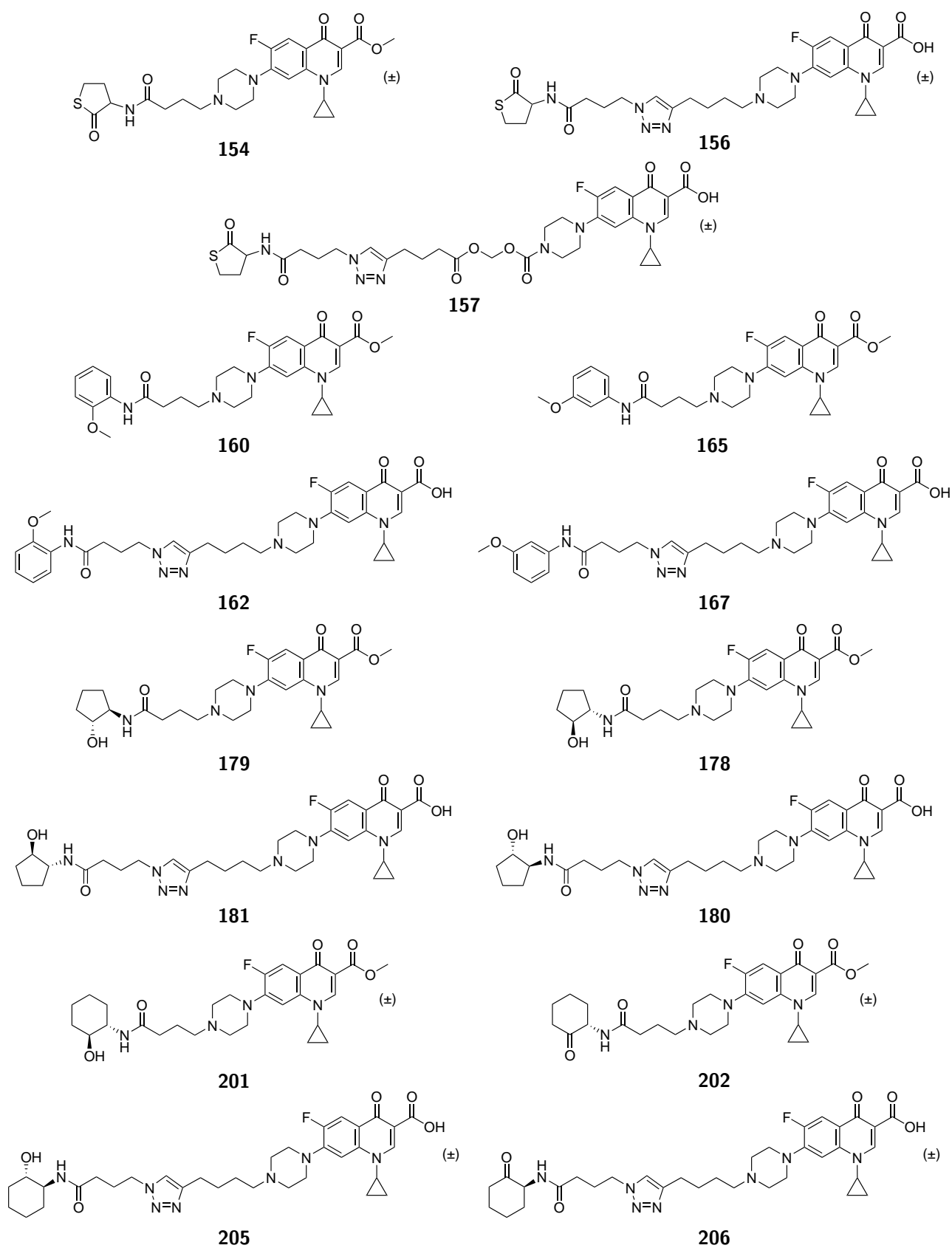


Figure 40: The HSL analogue-Cip(Me) conjugates.

## 8.7.1 Antibacterial testing

### 8.7.1.1 Antibacterial testing against YM64

In YM64 at 5 h several of the HSL analogue-Cip(Me) conjugates showed activity at the highest concentration (see Figure 41 and Figure 42). Conjugates **162** and **167** showed similar activity to ciprofloxacin **24** and the cleavable conjugate **157** showed better activity (see Figure 41). The activity of the cleavable conjugate **157** was even more pronounced at 24 h (see Figure 43).

It should be noted that the highest concentration tested was 25  $\mu\text{M}$  in this set of assays as opposed to 2  $\mu\text{M}$  in the previous set (see 7.5), but oddly all compounds including ciprofloxacin **24** showed less activity. This is thought to be due to a change in the plate seals used and/or the humidity of the incubation conditions (see 9.71).

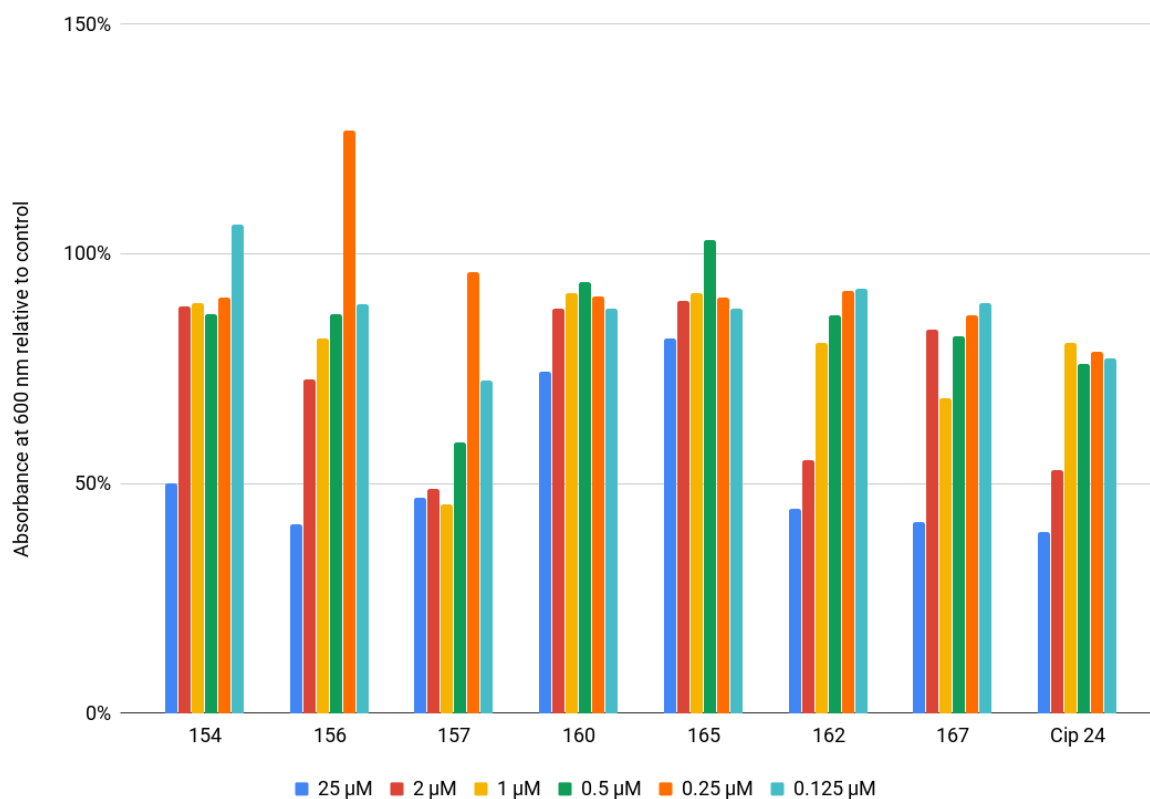


Figure 41: YM64 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

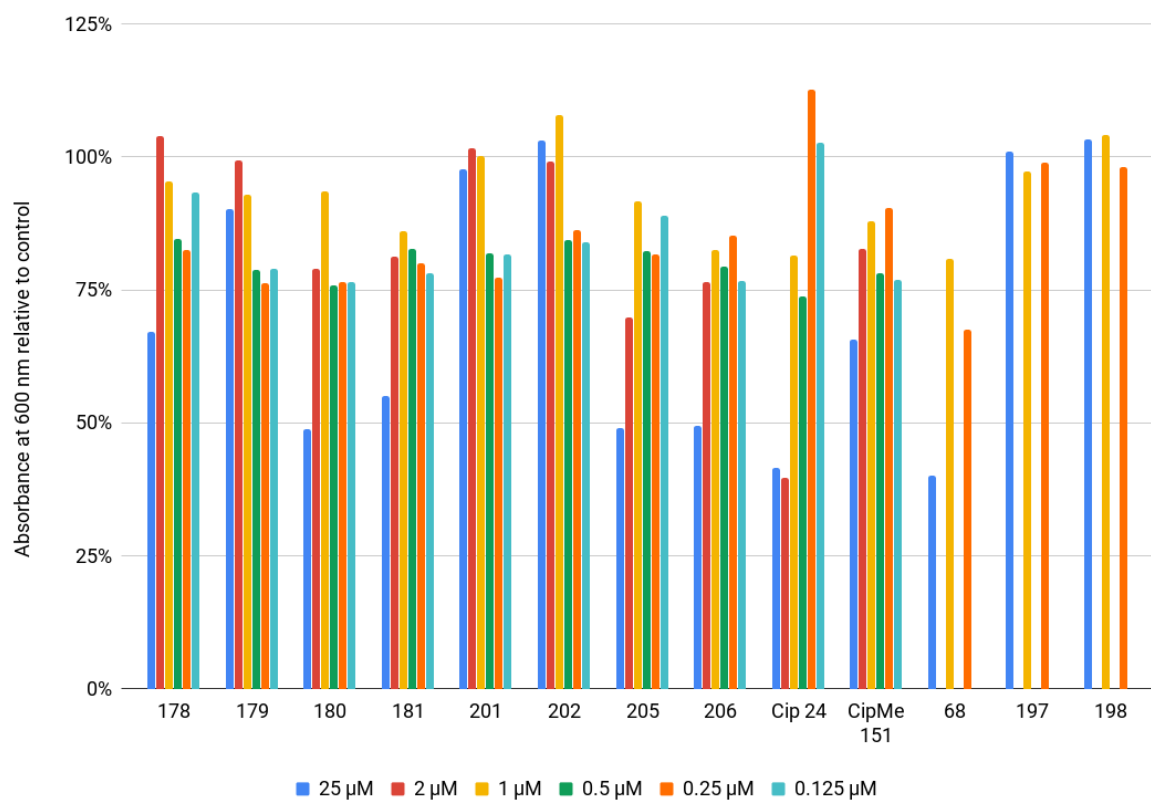


Figure 42: YM64 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

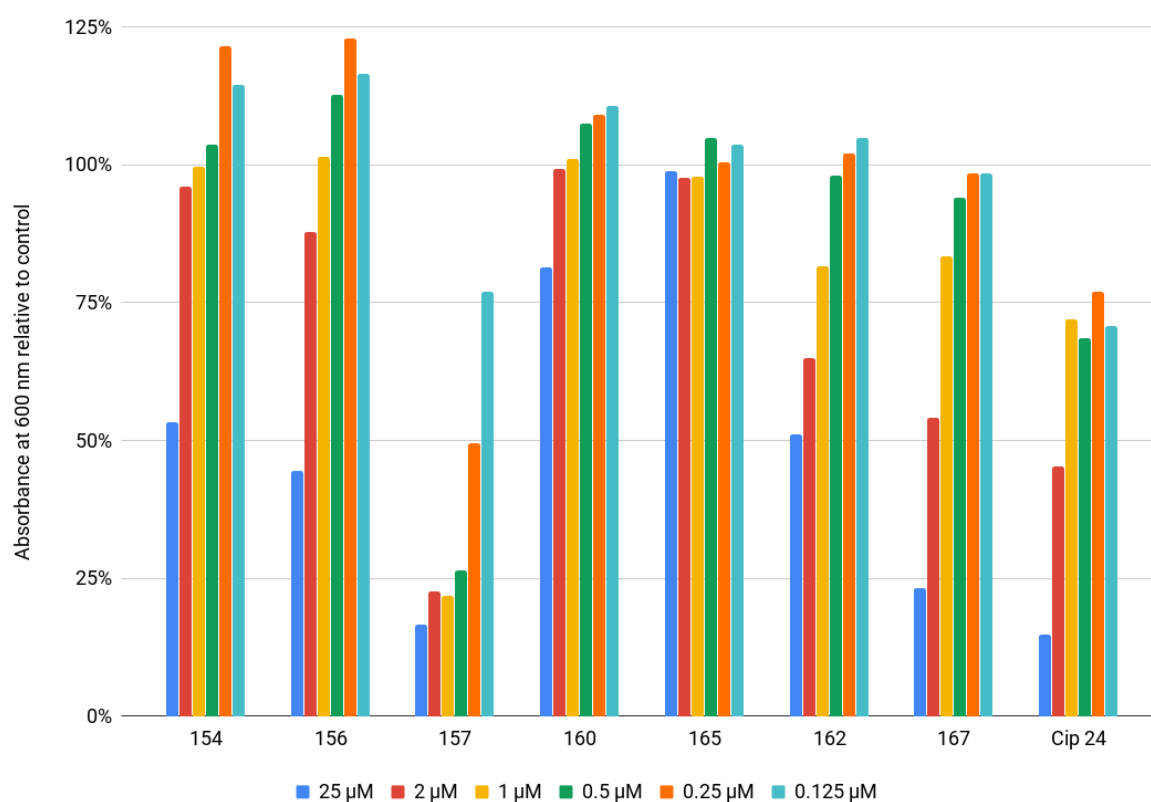


Figure 43: YM64 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

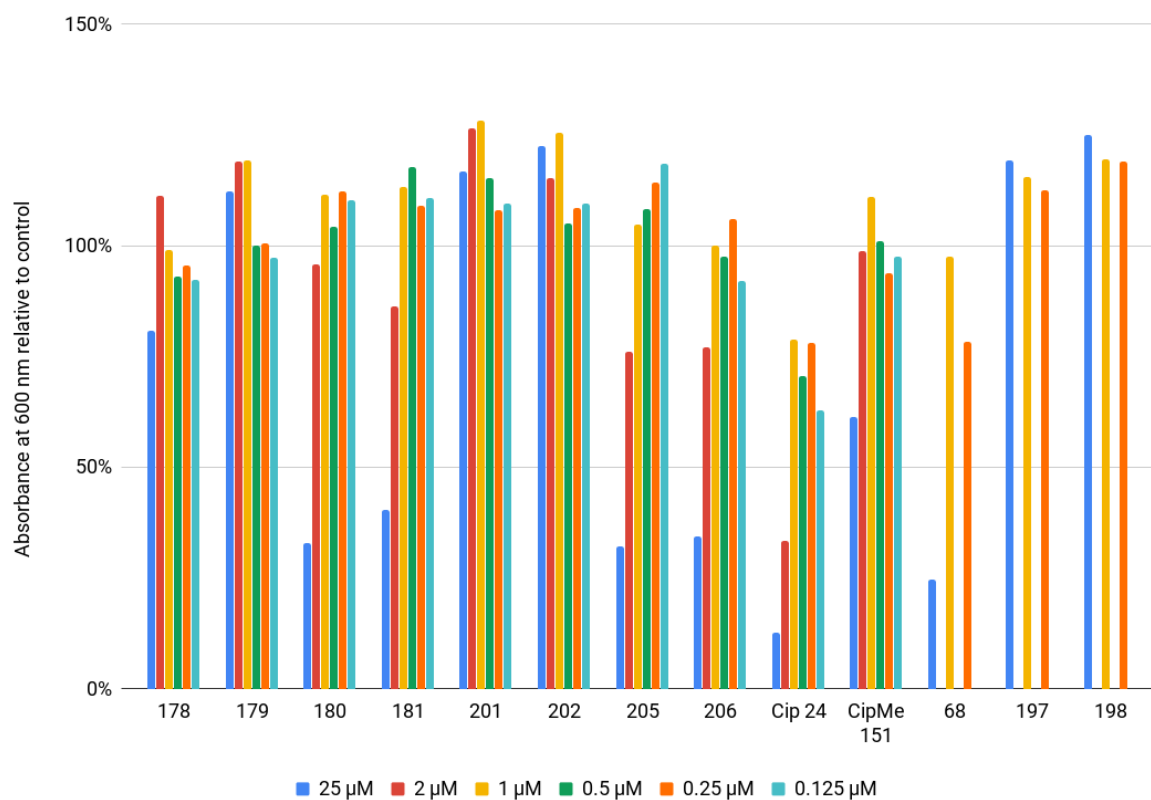


Figure 44: YM64 OD readings at 24 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

#### 8.7.1.2 Antibacterial testing against PAO1

In PAO1 at 5 h conjugates **157**, **162** and **167** showed activity at the highest concentration (see Figure 45). The cleavable conjugate **157** showed similar activity to ciprofloxacin **24**. At 24 h conjugate **167** still showed some activity, and cleavable conjugate **157** showed similar activity to ciprofloxacin **24** (see Figure 47).

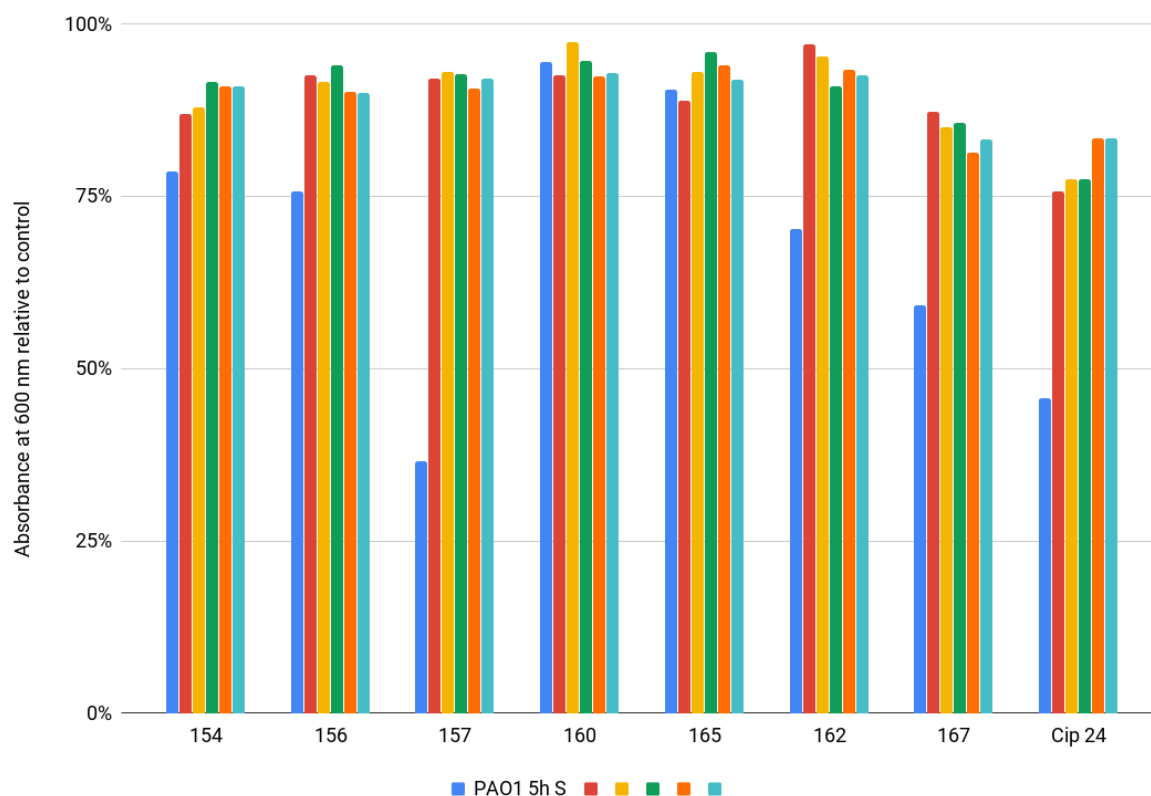


Figure 45: PAO1 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

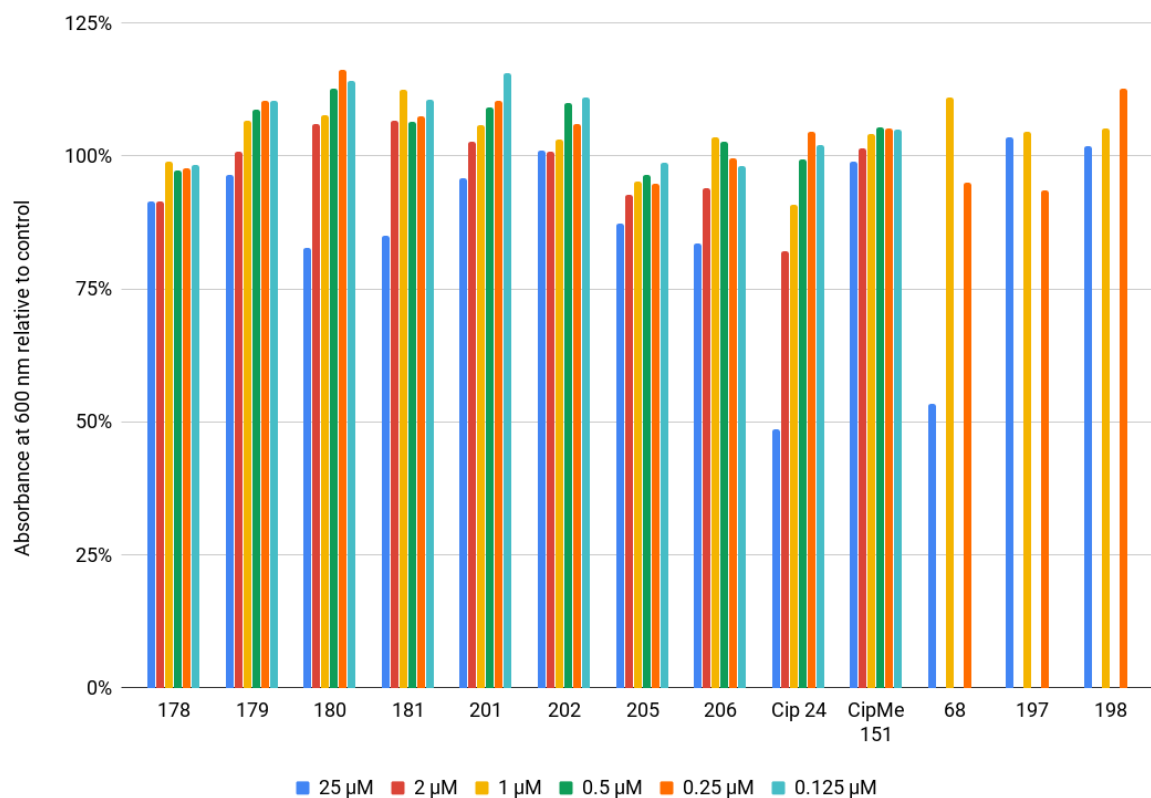


Figure 46: PAO1 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.



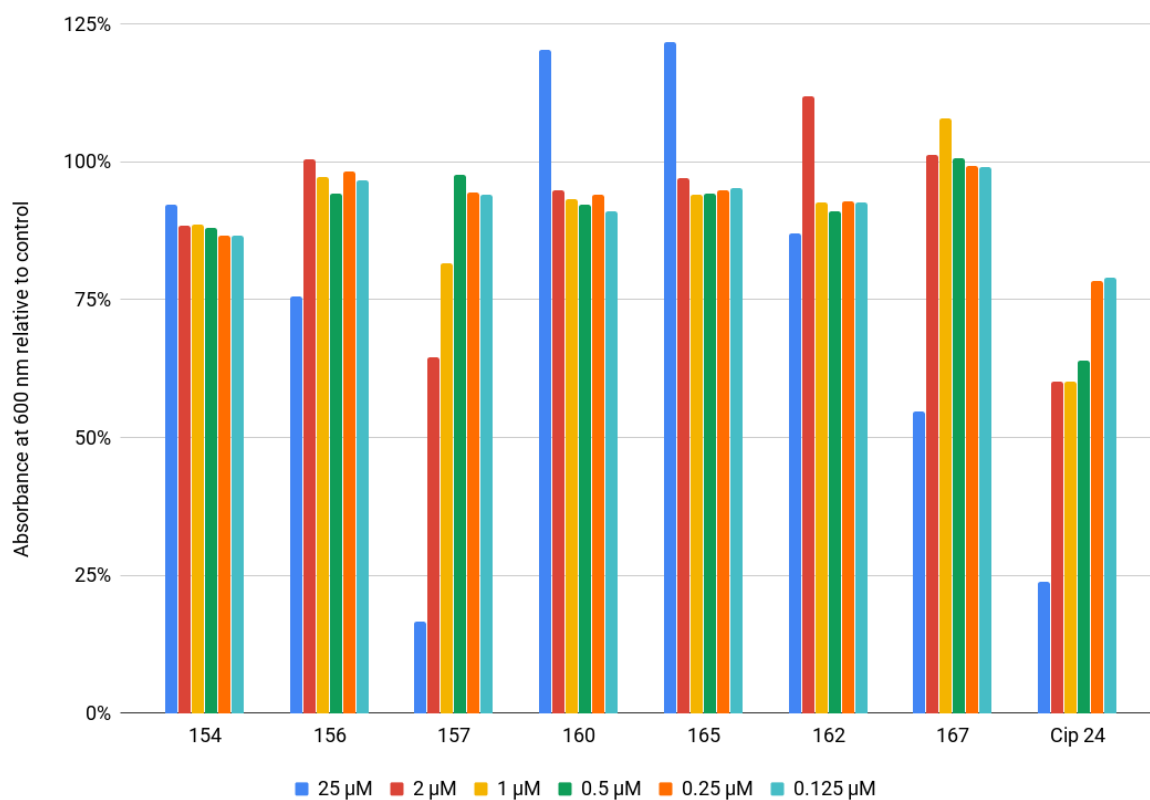


Figure 47: PAO1 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

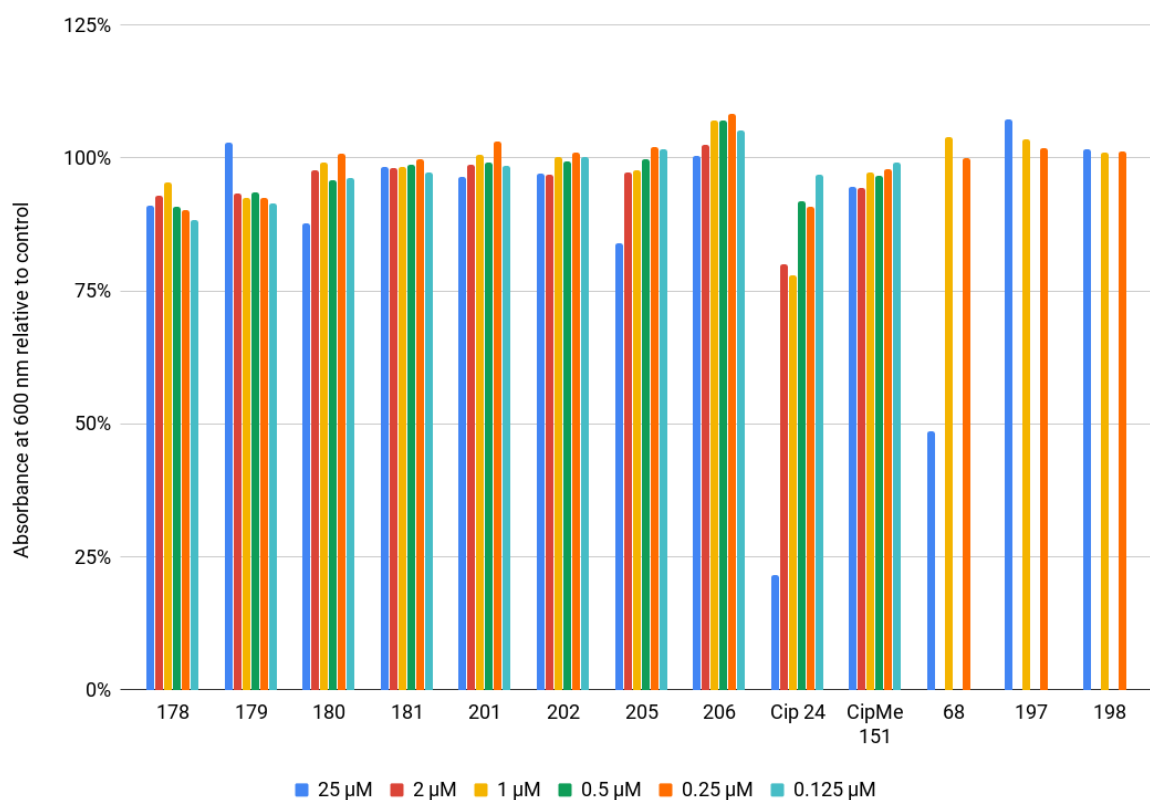


Figure 48: PAO1 OD readings at 24 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

In addition to its promising antibacterial activity, the cleavable HCTL-Cip triazole conjugate **157** has an

interesting growth curve (see Figure 49). When *P. aeruginosa* PAO1 is treated with 25  $\mu$ M ciprofloxacin **24** it continues to grow slowly over the course of a 48 h assay, whereas growth is fully inhibited by treatment with the cleavable HCTL-Cip triazole conjugate **157**. However, the errors in this data are large and so the assay needs repeating to confirm the effect.

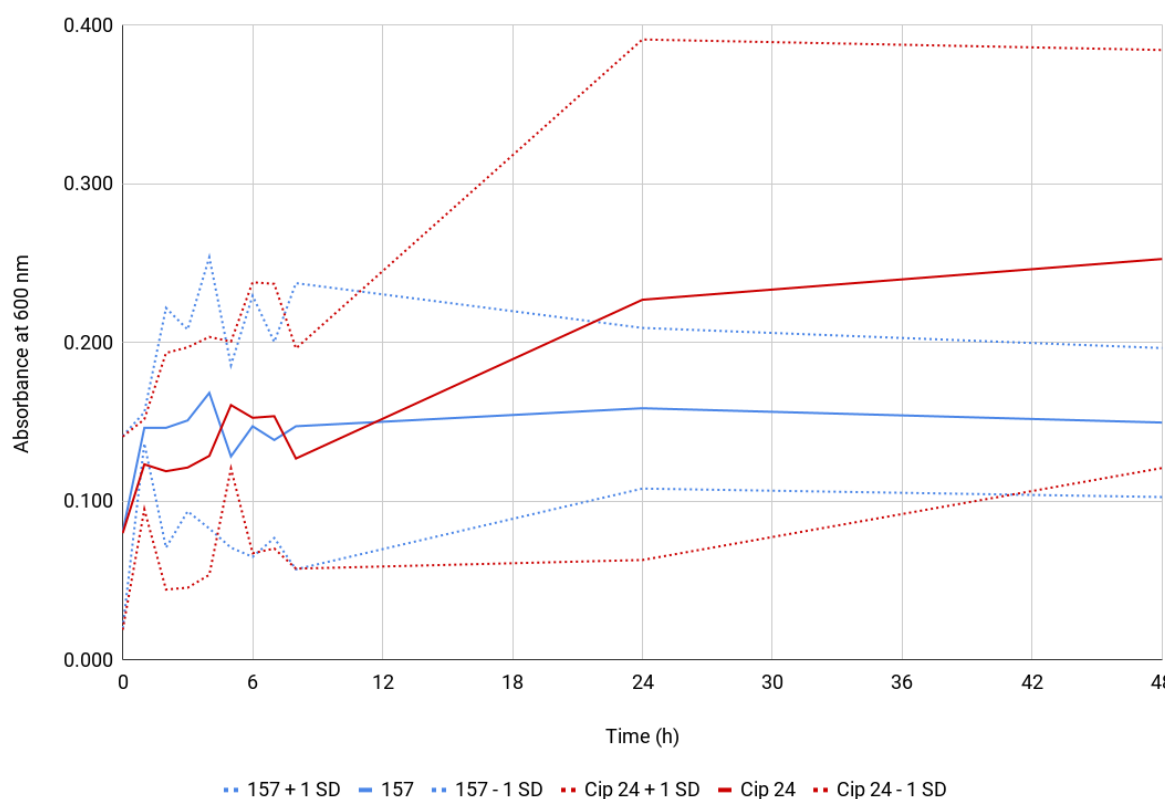


Figure 49: PAO1 OD readings over 48 h for the cleavable HCTL-Cip triazole conjugate **157** and ciprofloxacin **24** at 25  $\mu$ M.

### 8.7.2 Anti-biofilm testing

Biofilm inhibition and dispersal were measured using crystal violet staining (see 9.71). Unfortunately the results were largely unreliable as it was obvious that staining was higher in the wells at the edges of the plates, and that this was overwhelming any other trends. This effect was probably due to increased evaporation from the outer wells. It is likely that this effect was seen in these results, but not those in 7.5, due to a change in the conditions that the plates were incubated in. Specifically, a different type of plate seal was used and a humid environment was maintained in the incubator in the previous experiments (see 9.71).

## 8.8 Conclusions

### 8.8.1 Library synthesis

In this section, a library of HSL analogue-Cip(Me) conjugates was successfully synthesised and tested for antibiotic activity. A range of 7 head groups (see 8.1.1) and two linking strategies were used. Unfortunately the branching route that was initially proposed (see 8.1.2) was not feasible for the alcohol-containing head groups and was low yielding for others, probably due to internal cyclisation (this side reaction could with hindsight be avoided by changing the linker length).

Given the difficulties in the branching synthesis, routes to the differently-linked compounds were optimised

separately: the alkyl-linked conjugates were best formed using peptide coupling and the triazole-linked conjugates via a chloride intermediate. Direct comparisons of routes are not possible without repeating syntheses, but if it is assumed that peptide coupling of homocysteine thiolactone hydrochloride **152** to carboxylic acid **198** would have a similar yield to the coupling with (1*R*,2*R*)-2-aminocyclopentan-1-ol **173**, approximate comparisons can be made. The synthesis of the HCTL-CipMe conjugate **154** described in 8.2 has an overall yield of 11%, whereas the route to the cyclopentanol-CipMe conjugate **178** shown in Scheme 44 has an overall yield of 26%. Moreover, if the yield starting from the head group is considered, the yield is 55% vs. 11%. Therefore, the peptide coupling route is recommended for further investigation if the alkyl-linked library is to be expanded.

Synthesis of the azido autoinducer analogues via the chloride is also recommended as the bromide is thought to cyclise readily (this could explain the poor yields of the 2- and 3-methoxybenzene derivatives).

Preparative HPLC was identified as the best purification method for these conjugates (note that the standard acidic method used hydrolyses the lactone of native HSL and so cannot be used in that case).

### 8.8.2 Biology

The ciprofloxacin triazole conjugates had higher activity than the methyl ciprofloxacin conjugates. This was mirrored in the controls: methyl ciprofloxacin **151** showed little activity compared to ciprofloxacin **24**. It was assumed that methyl ciprofloxacin **151** would act as a prodrug, as the HCTL-CipMe conjugate **154** synthesised by Ganguly *et al.*<sup>61</sup> was a methyl ester and showed activity, but these results suggest otherwise. However, the HCTL-CipMe conjugate **154** showed better anti-biofilm activity than antibiotic, and it is possible that the other CipMe conjugates will do the same.

The most promising compounds from this set were the methoxybenzene-ciprofloxacin triazole conjugates **162** and **167** and the cleavable HCTL-Cip triazole conjugate **157**. The cleavable HCTL-Cip triazole conjugate **157** was also interesting in that it appeared to entirely inhibit the growth of *P. aeruginosa*, whereas ciprofloxacin **24** either allowed slow growth, or resistant mutants emerged and started to replicate.<sup>229</sup> However, the errors in these data were large and so the assays need to be repeated. Given the previous results for the cleavable HSL-Cip conjugates (see 7.5.2) is not very likely that the quorum sensing modulation properties of the head group contributed to its antibiotic effect, but the effect of different cleavable tails is certainly worth investigating.

Initial biofilm inhibition and dispersal assays were carried out (see 9.71), but unfortunately the results were unreliable as the biofilm was growing more in the wells at the edges of the plates. As biofilm formation is induced by hypoxia<sup>230</sup> (which might occur in the centre of the plate when a plastic lid was used in addition to the adhesive plate seal) this cannot account for the increased biofilm growth at the edges of the plate. Neither can the increased concentration of NaCl that would occur due to evaporation of water from the edges of the plate, as this too decreases biofilm formation.<sup>231</sup> A reasonable explanation is that evaporation would leave a residue of dried planktonic cells on the edges of the wells which would be stained by the crystal violet.

## 8.9 Future work

### 8.9.1 Further conjugates

An obvious addition to the library would be the HSL-CipMe conjugate (see Figure 50), to enable better comparisons between the triazole-linked and alkyl-linked libraries.

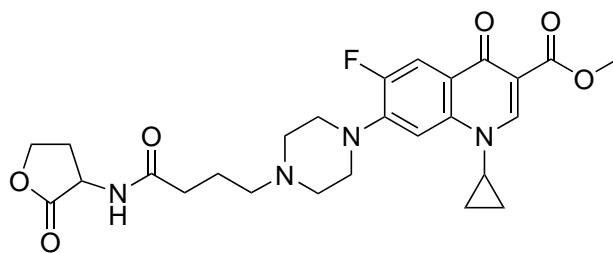


Figure 50: The proposed HSL-CipMe conjugate **207**.

As methyl ciprofloxacin **151** and its alkyl-linked conjugates had little antibiotic effect, it would be useful to test the carboxylic acid versions of these compounds (see Figure 51). Ideally these would be synthesised directly by hydrolysis of the methyl ciprofloxacin conjugate, but this could potentially cause hydrolysis of the lactone or thiolactone as well. If mild enough hydrolysis conditions could not be found it might be possible to hydrolyse both bonds and then re-form the ring.<sup>232</sup> The cyclohexanone conjugate would be best formed by hydrolysis of the cyclohexanol conjugate followed by oxidation of the alcohol to avoid exposing the ketone to extremes of pH.

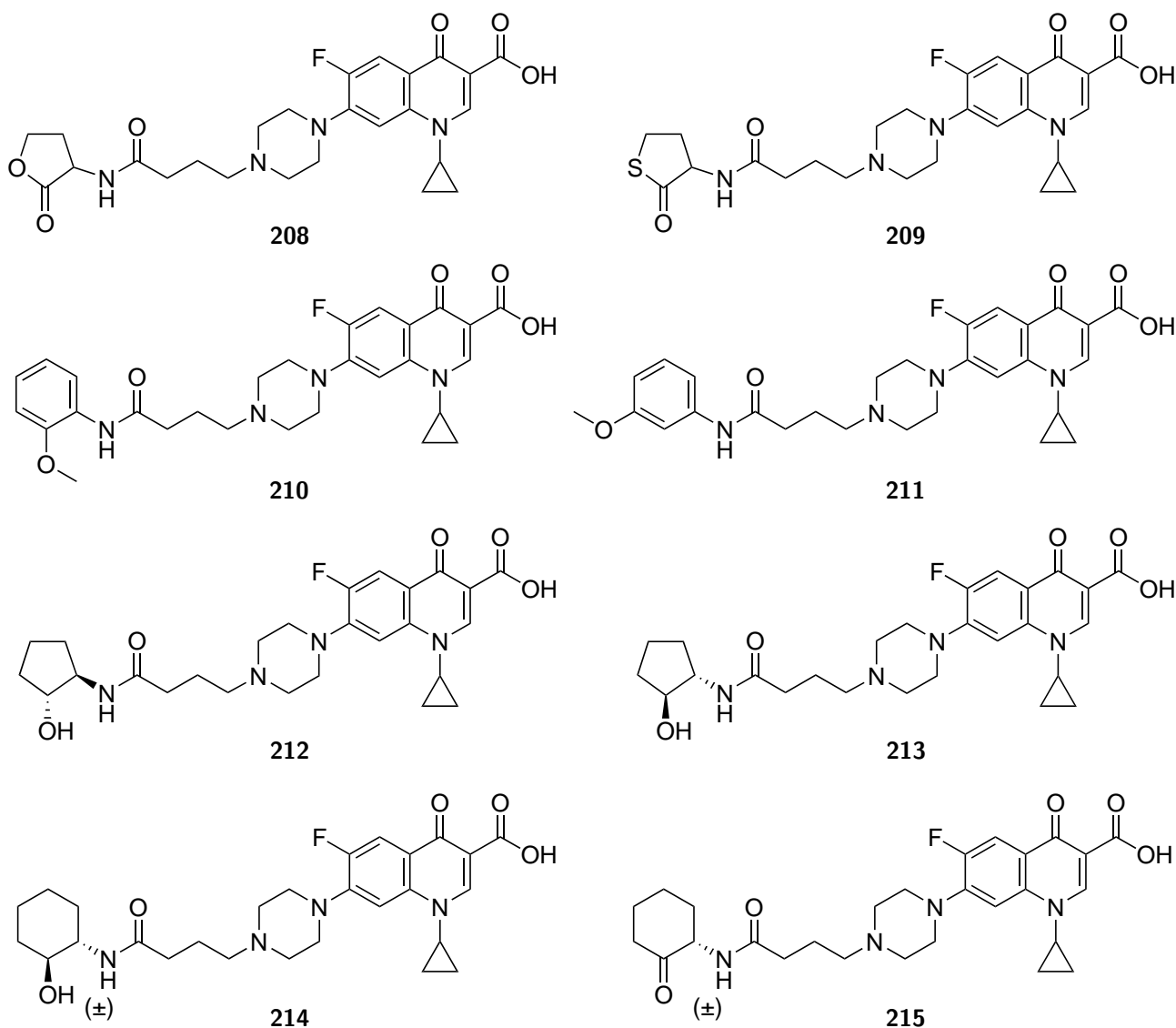


Figure 51: The proposed HSL analogue-Cip conjugates.

A selection head groups which could be used in future conjugates are shown in Figure 52. These have

all been shown to modulate HSL-mediated quorum sensing as part of acyl-HSLs.<sup>152, 157, 159, 233–236</sup> The most obvious targets are the cyclopentanone derivatives, as this could be synthesised from the alcohols above. The aniline, pyridine, quinoline and cyclopentyl amine head groups are commercially available and hence derivatives of these could be easily obtained. The 3- and 4-substituted HSL analogues require synthesis, but a route has been devised.<sup>235</sup>

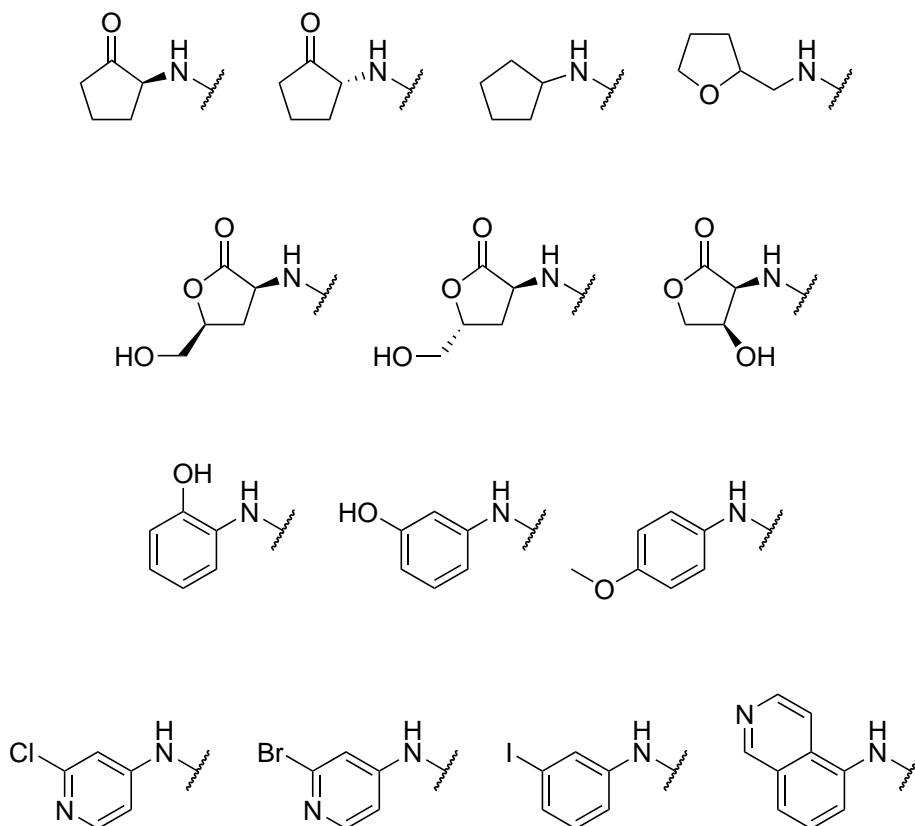


Figure 52: HSL analogue head groups for use in future conjugates.

### 8.9.2 Biology

The most important next step is the repetition of the biofilm inhibition and dispersal assays with better control over evaporation. This can be achieved in a low-tech but reliable manner by placing the sealed plates (without the plastic plate lids) inside a high-sided, open plastic box lined with damp tissue paper.

It is worth noting that Ganguly *et al.* used LIVE/DEAD® *BacLight*<sup>TM</sup> staining and confocal microscopy to image their biofilms, whereas so far we have used crystal violet staining. Crystal violet does not differentiate between live and dead cells, and so does not pick up on how many cells have been killed within the biofilm but stayed adhered to the plate (their confocal microscopy results do however show a decrease in biofilm thickness so it should be possible to detect this using crystal violet).

We do not have access to a confocal microscope on which we can use *P. aeruginosa*, but alternative stains which show cell viability could be used. Peeters *et al.*<sup>237</sup> evaluated six assays used for the quantification of biofilms and recommended fluorescein diacetate or resazurin viability assays for the quantification of live cells in *P. aeruginosa* biofilms.

Given the interesting growth curve results for the cleavable HCTL-Cip triazole conjugate **157** it would be useful to collect this data for the other cleavable compounds shown in 7.4.3 (previously OD readings were only taken at 5 and 24 h).

## 9 Experimental

### 9.1 General

Unless otherwise stated, reactions were performed in air-dried glassware under argon with dry, freshly-distilled solvents. THF was distilled from  $\text{LiAlH}_4$  in the presence of triphenyl methane indicator.  $\text{CH}_2\text{Cl}_2$ , hexane, MeOH and acetonitrile were distilled from calcium hydride. All other chemicals were used as obtained from commercial sources.

Reactions using microwave heating were performed in sealed vials using a CEM Discover SP microwave reactor.

Thin-layer chromatography (TLC) was performed using Merck pre-coated 0.23 mm thick plates of Keiselgel 60 F254 and visualised using UV ( $\lambda = 254$  or  $366$  nm) or by staining with  $\text{KMnO}_4$  or ninhydrin. All retention factors ( $R_f$ ) are given to 0.01. All column chromatography was carried out using Merck 9385 Keiselgel 60 silica gel (230-400 mesh) or using a CombiFlash<sup>®</sup> EZ Prep with RediSep<sup>®</sup> normal-phase silica flash columns. Preparative high-performance liquid chromatography (HPLC) was run on an Agilent 1260 Infinity machine, using a Supelcosil<sup>TM</sup> ABZ+PLUS column (250 mm  $\times$  21.2 mm, 5  $\mu\text{m}$ ) with a linear gradient system (solvent A: 0.025% (*v/v*) TFA/water, solvent B: 0.05% (*v/v*) TFA/acetonitrile) at a flow rate of 20 mL min<sup>-1</sup>, visualised by UV absorbance ( $\lambda_{\text{max}} = 254$  nm)

Nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock at ambient probe temperatures on Bruker DPX-400, Bruker Avance DRX-400, Bruker Avance 500 BB-ATM or Bruker Avance 500 Cryo Ultrashield spectrometers. Data were processed using NMR Processor Academic Edition version 12 (ADC Labs) or TopSpin version 3.5 (Bruker). <sup>1</sup>H and <sup>13</sup>C spectra were assigned using DEPT, COSY, HMQC and HMQC spectra where necessary, or by analogy to fully interpreted spectra of related compounds. The following abbreviations are used to indicate the multiplicity of signals: s singlet, d doublet, t triplet, q quartet, quin quintet, m multiplet and br broad.

<sup>1</sup>H chemical shifts ( $\delta$ ) are quoted to the nearest 0.01 ppm and are referenced relative to the residual solvent peak.<sup>238</sup> Coupling constants ( $J$ ) are given to the nearest 0.1 Hz. Diastereotopic protons are assigned as  $\text{CHH}$  and  $\text{CHH}$ , where the latter designates the lower-field proton. Data are reported as follows: <chemical shift> (<multiplicity>, <coupling constant(s) (if any)>, <integration>, <assignment>).

<sup>13</sup>C chemical shifts ( $\delta$ ) are quoted to the nearest 0.1 ppm and are referenced relative to the deuterated solvent peak.<sup>238</sup> Data are reported as follows: <chemical shift> (<multiplicity (if not s)>, <coupling constant(s) (if any)>, <assignment>).

<sup>19</sup>F chemical shifts ( $\delta$ ) are quoted to the nearest 0.1 ppm. Data are reported as follows: <chemical shift> (<assignment>).

High resolution mass spectrometry (HRMS) data were recorded using a Micromass LCT Premier spectrometer or a Waters Vion IMS-QTOF spectrometer and reported mass values are within  $\pm 5$  ppm mass units. Liquid chromatography-mass spectrometry (LCMS) data were recorded on an Agilent 1200 series LC with an ESCi Multi-Mode Ionisation Waters ZQ spectrometer or a Waters ACQUITY H-Class UPLC with an ESCi Multi-Mode Ionisation Waters SQD2 mass spectrometer.

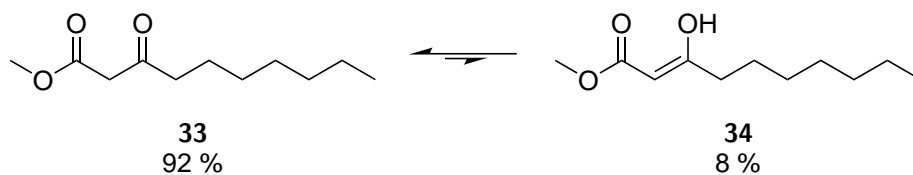
Infrared (IR) spectra were recorded using neat sample on a PerkinElmer 1600 FT IR spectrometer. Selected absorption maxima ( $\nu_{\text{max}}$ ) are reported in wavenumbers (cm<sup>-1</sup>). Broad peaks are marked br.

Melting points (m.p.) were measured using a Buchi B-545 melting point apparatus and are uncorrected.

Optical rotations ( $[\alpha]_D^T$ ) were recorded on a PerkinElmer 343 polarimeter or an Anton-Paar MCP 100 polarimeter.  $[\alpha]_D^T$  values are reported in  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  at 589 nm and concentration ( $c$ ) is given in g (100 mL)<sup>-1</sup>.

All compounds subjected to biological testing were >95% pure unless otherwise stated.

## 9.2 Methyl 3-oxodecanoate **33**



Meldrum's acid **31** (9.00 g, 63.0 mmol, 1 eq.) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (150 mL) in an oven-dried flask and cooled to 0 °C. Pyridine (10.2 mL, 126 mmol, 2 eq.) was added dropwise over 20 min. Octanoyl chloride **32** (11.7 mL, 69.0 mmol, 1.1 eq.) was then added and the mixture was stirred at 0 °C for a further 4 h. The mixture was allowed to warm to r.t., diluted with  $\text{CH}_2\text{Cl}_2$  (20 mL) and poured into a mixture of ice (~30 g) and HCl (2 N, 90 mL). The solution was washed with NaCl (sat., aq., 150 mL) and dried over  $\text{MgSO}_4$ . The solvent was removed under vacuum to give an orange-brown oil. The oil was refluxed in anhydrous MeOH (150 mL) for 5 h and the solvent was removed under vacuum. The resulting residue was purified by column chromatography ( $\text{SiO}_2$ , 5%  $\text{Et}_2\text{O}$ /40-60 P.E.). A tautomeric mixture of **33** and **34** was obtained as a colourless oil (8.34 g, 41.6 mmol, 66%. 92% **33** as determined by  $^1\text{H}$  NMR).

### Keto form **33**

**TLC**  $R_f$  = 0.12 (5%  $\text{EtO}_2$ /PE)

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2928 (C-H), 2856 (C-H), 1747 (ester C=O), 1717 (ketone C=O)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 3.74 (s, 3 H,  $\text{OCH}_3$ ), 3.45 (s, 2 H,  $\text{C(=O)CH}_2\text{C(=O)}$ ), 2.53 (t,  $J$  = 7.4 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ ), 1.60 (quin,  $J$  = 7.1 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ ), 1.39 - 1.19 (m, 8 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.88 (t,  $J$  = 6.8 Hz, 3 H,  $\text{CH}_2\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 202.3 ( $\text{CH}_3\text{OC(=O)CH}_2\text{C(=O)}$ ), 167.3 ( $\text{CH}_3\text{OC(=O)CH}_2\text{C(=O)}$ ), 51.7 ( $\text{OCH}_3$ ), 48.5 ( $\text{CH}_3\text{OC(=O)CH}_2\text{C(=O)}$ ), 42.5 ( $\text{C(=O)CH}_2\text{CH}_2$ ), 31.3 ( $\text{CH}_2$ ), 28.7 ( $\text{CH}_2$ ), 28.6 ( $\text{CH}_2$ ), 23.1 ( $\text{CH}_2$ ), 22.2 ( $\text{CH}_2$ ), 13.6 ( $\text{CH}_2\text{CH}_3$ )

### Enol form **34**

**TLC**  $R_f$  = 0.12 (5%  $\text{EtO}_2$ /PE)

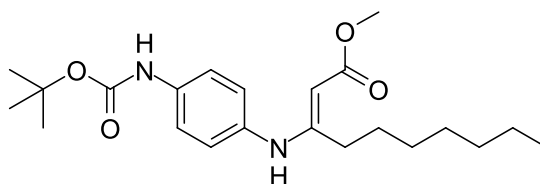
**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2928 (C-H), 2856 (C-H), 1654 (C=C), 1629 ( $\alpha,\beta$  unsaturated C=O)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 12.02 (s, 1 H,  $\text{COH}$ ), 4.99 (s, 1 H,  $\text{C(=O)CH=COH}$ ), 3.73 (s, 3 H,  $\text{OCH}_3$ ), 2.20 (t,  $J$  = 7.4 Hz, 2 H,  $\text{COHCH}_2$ ), 1.76 - 1.72 (m, 2 H,  $\text{COHCH}_2\text{CH}_2$ ), 1.39 - 1.19 (m, 8 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.88 (t,  $J$  = 6.8 Hz, 3 H,  $\text{CH}_2\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 178.7 ( $\text{CH}_3\text{OC(=O)CH=COH}$ ), 172.7 ( $\text{CH}_3\text{OC(=O)CH=COH}$ ), 88.2 ( $\text{CH}_3\text{OC(=O)CH=COH}$ ), 50.5 ( $\text{OCH}_3$ ), 37.9 ( $\text{COHCH}_2\text{CH}_2$ ), 34.6 ( $\text{CH}_2$ ), 31.2 ( $\text{CH}_2$ ), 29.0 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 22.3 ( $\text{CH}_2$ ), 13.6 ( $\text{CH}_2\text{CH}_3$ )

Spectroscopic data are consistent with the literature.<sup>168,169</sup>

### 9.3 Methyl (*E*)-3-((4-((*tert*-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate **36**



Methyl 3-oxodecanoate **33** (500 mg, 2.50 mmol, 1.00 eq.) and *O*-*tert*-butyl *N*-(4-aminophenyl)carbamate **35** (520 mg, 2.50 mmol, 1.00 eq.) were dissolved in MeOH (10 mL) and refluxed for 18 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (SiO<sub>2</sub>, gradient of 0 to 20% Et<sub>2</sub>O/40-60 P.E.). **36** was obtained as a white amorphous solid (0.169 mg, 0.480 mmol, 19%).

**TLC**  $R_f$  = 0.30 (30% Et<sub>2</sub>O/40-60 P.E.)

**mp**  $T$  / °C = 79 (Et<sub>2</sub>O/40-60 P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3337 (N-H), 2928 (C-H), 2857 (C-H), 1724 (carbamate C=O), 1635 ( $\alpha,\beta$  unsaturated C=O), 1611 (C=C), 1581 (N-H bend)

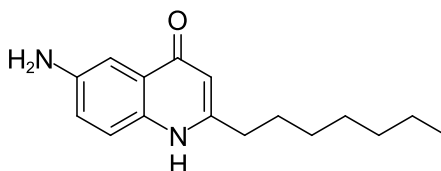
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 10.16 (s, 1 H, NHC(C<sub>7</sub>H<sub>15</sub>)=C), 7.35 (d,  $J$  = 8.6 Hz, 2 H, *meta* to NHBoc), 7.02 (d,  $J$  = 8.7 Hz, 2 H, *meta* to enamine), 6.60 (br s, 1 H, NHBoc), 4.71 (s, 1 H, C=CH), 3.70 (s, 3 H, OCH<sub>3</sub>), 2.23 (t,  $J$  = 7.7 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.54 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.40 (quin,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.33 - 1.16 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.86 (t,  $J$  = 7.1 Hz, 3 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 171.1 (C(=O)CH=C), 164.3 (C(=O)CH=C), 152.7 (OC(=O)NH), 136.0 (*para* to NHBoc), 134.1 (CNHBoc), 126.3 (*meta* to NHBoc), 119.1 (*ortho* to NHBoc), 83.8 (C(=O)CH=C), 80.7 (C(CH<sub>3</sub>)<sub>3</sub>), 50.2 (OCH<sub>3</sub>), 32.2 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 28.0 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 14.0 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 391.2589, [M+H]<sup>+</sup>, [C<sub>22</sub>H<sub>35</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup> requires 391.2591

Spectroscopic data are consistent with the literature.<sup>164</sup>

### 9.4 6-Amino-2-heptylquinolin-4-ol **37**



Methyl (*E*)-3-((4-((*tert*-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate **36** (168 mg, 0.649 mmol, 1 eq.) and polyphosphoric acid (5 g) were heated to 90 °C for 1 h. The reaction mixture was then poured into NaHCO<sub>3</sub> (sat., aq., 50 mL) cooled with ice. The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum. **37** was obtained as a pale yellow amorphous solid (121 mg, 0.468 mmol,



72%).

mp  $T / ^\circ\text{C} = 249$  (water)

IR (neat)  $\nu_{\max} / \text{cm}^{-1} = 3337$  (N-H), 2927 (C-H), 2857 (C-H), 1635 (C=O)

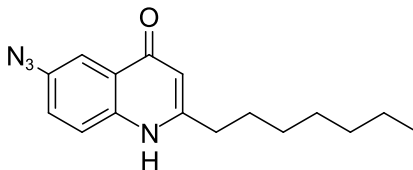
$^1\text{H}$  NMR (400 MHz, DMSO- $\text{d}_6$ )  $\delta / \text{ppm} = 7.26$  (d,  $J = 8.7$  Hz, 1 H, *meta* to  $\text{NH}_2$ ), 7.15 (d,  $J = 2.6$  Hz, 1 H, *ortho* to C(=O)), 6.95 (dd,  $J = 2.7, 8.8$  Hz, 1 H, *para* to C(=O)), 5.74 (s, 1 H, *ortho* to  $\text{CH}_2$ ), 5.16 (s, 2 H,  $\text{NH}_2$ ), 2.52 (t,  $J = 7.4$  Hz, 2 H,  $\text{CCH}_2$ ), 1.64 (quin,  $J = 7.6$  Hz, 2 H,  $\text{CCH}_2\text{CH}_2$ ), 1.36 - 1.19 (m, 8 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.86 (t,  $J = 7.0$  Hz, 3 H,  $\text{H}_3$ )

$^{13}\text{C}$  NMR (101 MHz, DMSO- $\text{d}_6$ )  $\delta / \text{ppm} = 176.7$  (C(=O)), 151.7 ( $\text{CCH}_2$ ), 145.1 (*para* to  $\text{NH}_2$  or *ipso* to C(=O)), 132.4 (*ipso* to  $\text{NH}_2$ ), 126.6 (*para* to  $\text{NH}_2$  or *ipso* to C(=O)), 121.1 (*para* to C(=O)), 119.0 (*meta* to  $\text{NH}_2$  and *meta* to C(=O)), 106.2 ( $\text{CH}=\text{CCH}_2$ ), 105.9 (*ortho* to  $\text{NH}_2$  and *ortho* to C(=O)), 33.6 ( $\text{CCH}_2$ ), 31.6 ( $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 29.0 ( $\text{CH}_2$ ), 29.0 ( $\text{CH}_2$ ), 28.9 ( $\text{CH}_2$ ), 22.5 ( $\text{CH}_2\text{CH}_3$ ), 14.4 ( $\text{CH}_3$ )

HRMS (ESI $^+$ )  $m/z / \text{Da} = 259.1810$ ,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}]^+$  requires 259.1803

Spectroscopic data are consistent with the literature.<sup>164</sup>

## 9.5 6-Azido-2-heptylquinolin-4-ol **38**



6-Amino-2-heptylquinolin-4-ol **37** (50 mg, 0.194 mmol, 1 eq) was dissolved in HCl (conc., aq., 1.20 mL), water (1.80 mL) and MeOH (2.00 mL) and cooled to 0  $^\circ\text{C}$ . A solution of  $\text{NaNO}_2$  (16.0 mg, 0.232 mmol, 1.2 eq.) in water (0.300 mL) was added dropwise over 10 min and the mixture was stirred for 1 h. A solution of  $\text{NaN}_3$  (15.1 mg, 0.232 mmol, 1.2 eq.) in water (0.300 mL) was then added. The mixture was warmed to room temperature and stirred for a further 4 h. The resultant precipitate was filtered off and dried under reduced pressure. **38** hydrochloride salt\* was obtained as a pale cream amorphous solid (25.6 mg, 0.0800 mmol, 41%).

TLC  $R_f = 0.40$  (5% MeOH/ $\text{CH}_2\text{Cl}_2$ )

IR (neat)  $\nu_{\max} / \text{cm}^{-1} = 3249$  (N-H), 3065 (N-H), 2917 (C-H), 2853 (C-H), 2728 (C-H), 2107 (azide), 1635 (C=O)

$^1\text{H}$  NMR (400 MHz, MeOD)  $\delta / \text{ppm} = 7.73$  (d,  $J = 8.6$  Hz, 1 H, *ortho* to NH), 7.71 (d,  $J = 2.8$  Hz, 1 H, *ortho* to  $\text{N}_3$  and *ortho* to C(=O)), 7.47 (dd,  $J = 8.9, 2.7$  Hz, 1 H, *para* to C(=O)), 6.24 (s, 1 H, C(=O) $\text{CH}$ ), 2.69 (t,  $J = 7.7$  Hz, 2 H,  $\text{CCH}_2$ ), 1.68 (quin,  $J = 7.6$  Hz, 2 H,  $\text{CCH}_2\text{CH}_2$ ), 1.28 - 1.39 (m, 4 H,  $\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 1.18 - 1.28 (m, 4 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.85 (t,  $J = 6.8$  Hz, 3 H,  $\text{CH}_3$ )

$^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta / \text{ppm} = 172.3$  (C(=O)), 155.5 ( $\text{NHCCH}_2$ ), 137.4 ( $\text{CN}_3$ ), 135.6 (*para* to  $\text{N}_3$ ), 124.6 (*para* to C(=O)), 124.1 (*ipso* to C(=O)), 120.7 (*meta* to  $\text{N}_3$  and *meta* to C(=O)), 112.8 (*ortho* to  $\text{N}_3$  and *or-*

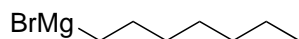
*tho* to C(=O)), 107.0 (C(=O)CH), 33.3 (NHCCH<sub>2</sub>), 31.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.3 - 28.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 22.1 (CH<sub>2</sub>CH<sub>3</sub>), 14.0 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 285.1728, [M+H]<sup>+</sup> found, [C<sub>16</sub>H<sub>21</sub>N<sub>4</sub>O]<sup>+</sup> requires 285.1715

Spectroscopic data are similar to the literature characterisation of the free amine.<sup>164</sup>

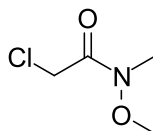
\*Probably as the 4-hydroxyquinoline.<sup>89</sup>

## 9.6 Heptyl magnesium bromide 40



Magnesium turnings (352 mg, 14.5 mmol, 1 eq.) were added to an oven-dried flask under argon. THF (15 mL) was added, followed by bromoheptane **39** (2.40 mL, 14.5 mmol, 1 eq.) dropwise. The mixture was stirred at r.t. for 2 h followed by heating to reflux for 2 h. Heptyl magnesium bromide **40** was obtained as a pale grey suspension (15 mL, ~ 1 M) which was used without further purification.

## 9.7 2-Chloro-*N*-methoxy-*N*-methylacetamide 43



*N*,*O*-Dimethylhydroxyl amine hydrochloride **41** (6.00 g, 61.5 mmol, 1 eq.) and toluene (75 mL) were added successively to a stirred solution of potassium carbonate (22.4 g, 162 mmol, 2.63 eq.) in water (75 mL) at 0 °C under argon. The mixture was cooled to -5 °C and chloroacetyl chloride **42** (5.88 mL, 73.8 mmol, 1.20 eq.) was added dropwise over 5 min. The mixture was allowed to warm to r.t. over 30 min, then the organic layer was separated and the aqueous layer was extracted with toluene (3×20 mL). The combined organic extracts were dried with MgSO<sub>4</sub> and the solvent was removed by rotary evaporation followed by high vacuum. **43** was obtained as white, prism-like crystals (7.24 g, 52.6 mmol, 71%).

**mp**  $T$  / °C = 39 (toluene)

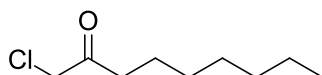
**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3016.7 (C-H), 2966.4 (C-H), 2946.7 (C-H), 2827.7 (C-H), 1666.2 (C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 4.20 (s, 2 H, ClCH<sub>2</sub>C=O), 3.71 (m, 3 H, OCH<sub>3</sub>), 3.18 (s, 3 H, NCH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 167.4 (C=O), 61.6 (OCH<sub>3</sub>), 40.9 (ClCH<sub>2</sub>C=O), 32.6 (NCH<sub>3</sub>)

Spectroscopic data are consistent with the literature.<sup>89</sup>

## 9.8 1-Chlorononan-2-one 44



2-Chloro-*N*-methoxy-*N*-methylacetamide **43** (1.00 g, 7.26 mmol, 1 eq.) was added to a dry flask under argon. THF (20 mL) was added and the flask cooled to 0 °C. Heptyl magnesium bromide **40** (~ 1 M, 15.0 mL, 15.0 mmol, 2.07 eq.) was added dropwise over 5 min, then the mixture was allowed to warm to r.t. and stirred for 15 h. The reaction mixture was then poured into HCl (aq., 2 N, 60 mL) at 0 °C and stirred for 10 min. The mixture was extracted with toluene (30 mL) and the aqueous layer discarded. The organic layer was washed with brine and dried with MgSO<sub>4</sub>, and the solvent was removed by rotary evaporation. **44** was obtained as a colourless oil (1.23 g, 6.96 mmol, 96%).

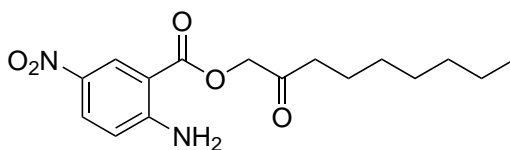
**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2952 (C-H), 2925 (C-H), 2856 (C-H), 1720 (C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 4.05 (s, 2 H, ClCH<sub>2</sub>C(=O)), 2.54 (t, *J* = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.59 (quin, *J* = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.34 - 1.21 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.87 (t, *J* = 6.8 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 202.6 (C(=O)), 48.1 (CH<sub>2</sub>Cl), 39.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 31.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.9 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 23.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 13.9 (CH<sub>3</sub>)

Spectroscopic data are consistent with the literature.<sup>89</sup>

## 9.9 2-Oxononyl 2-amino-5-nitrobenzoate **46**



5-Nitroanthranilic acid **45** (500 mg, 2.75 mmol, 1.38 eq.) and potassium carbonate (270 mg, 2.00 mmol, 1 eq.) were dissolved in DMF (5 mL). The mixture was heated under argon to 90 °C and stirred for 1 h then cooled to r.t.. 1-Chlorononan-2-one **44** (353 mg, 2.00 mmol, 1 eq.) was added and the mixture was stirred for 15 h. The solution was poured into Na<sub>2</sub>HCO<sub>3</sub> (aq., 10%, 50 mL) and ice (~ 20 g). The precipitate was collected by vacuum filtration, washed with water and dried under high vacuum. **46** was obtained as a yellow amorphous solid (0.674 g, 2.00 mmol, >99%).

**mp** *T* / °C = 135 (water)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3453 (N-H), 3351 (N-H), 2925 (C-H), 2854 (C-H), 1720 (ester C=O) 1704 (ketone C=O) 1626 (N-H bend) 1603 (aromatic) 1573 (N-O) 1507 (N-O)

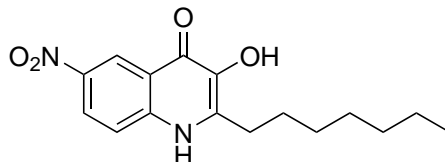
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 8.66 (d, *J* = 2.8 Hz, 1 H, *ortho* to C(=O)), 8.12 (dd, *J* = 2.8, 9.4 Hz, 1 H, *para* to C(=O)), 6.93 (d, *J* = 9.4 Hz, 1 H, *meta* to C(=O)), 5.05 (s, 2 H, OCH<sub>2</sub>C(=O)), 2.49 (t, *J* = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin, *J* = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.32 - 1.20 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.86 (t, *J* = 6.8 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 204.4 (OCH<sub>2</sub>C(=O)), 165.6 (C(=O)O), 156.3 (*ipso* to NH<sub>2</sub>), 135.7 (*ipso* to NO<sub>2</sub>), 129.6 (*para* to C(=O)), 128.9 (*ortho* to C(=O)), 117.4 (*meta* to C(=O)), 107.5 (*ipso* to C(=O)), 68.8 (OCH<sub>2</sub>C(=O)), 38.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.9 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 23.2 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 14.4 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 323.1610, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>23</sub>N<sub>2</sub>O<sub>5</sub>]<sup>+</sup> requires 323.1607

Spectroscopic data are consistent with the literature.<sup>164</sup>

### 9.10 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **47**



2-Oxononyl 2-amino-5-nitrobenzoate **46** (100 mg, 0.340 mmol, 1 eq.) and polyphosphoric acid (300 mg) were stirred for 5.5 h at 90 °C under argon. The mixture was then poured into NaHCO<sub>3</sub> (sat., aq., 50 mL) cooled on ice. The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum. **47** was obtained as a yellow-brown amorphous solid (44 mg, 0.145 mmol, 43%).

**mp**  $T$  / °C = 223 (water, EtOAc)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3436 (N-H), 3000 (O-H, br), 2955 (C-H), 2926 (C-H), 2851 (C-H), 1648 (C=O), 1571 (N-O), 1536 (N-O)

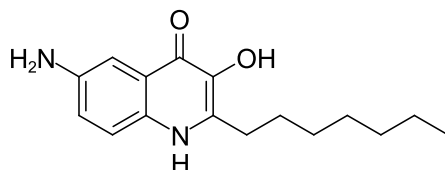
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 12.00 (s, 1 H, NH), 8.91 (d,  $J$  = 2.8 Hz, 1 H, *ortho* to C=O), 8.29 (dd,  $J$  = 2.7, 9.2 Hz, 1 H, *para* to C=O), 7.70 (d,  $J$  = 9.3 Hz, 1 H, *meta* to C=O), 2.75 (t,  $J$  = 7.7 Hz, 2 H, CCH<sub>2</sub>), 1.67 (quin,  $J$  = 7.3 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 1.36 - 1.23 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.85 (t,  $J$  = 7.0 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 169.7 (C=O), 141.9 (*para* to NO<sub>2</sub>), 140.7 (*ipso* to NO<sub>2</sub>), 139.6 (*ipso* to OH), 137.3 (C=COH), 124.3 (*para* to C=O), 122.3 (*ortho* to NO<sub>2</sub> and *ortho* to C=O), 121.5 (*ipso* to C=O), 120.0 (*meta* to NO<sub>2</sub> and *meta* to C=O), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 29.2 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 28.5 (CCH<sub>2</sub>), 28.1 (CCH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 14.4 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 305.1501, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>21</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup> requires 305.1500

Spectroscopic data are consistent with the literature.<sup>164</sup>

### 9.11 6-Amino-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **48**



6-Nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **47** (20 mg, 0.0658 mmol, 1 eq.) and PtO<sub>2</sub> (2 mg, 10 weight %) were stirred in MeOH (1 mL) under a H<sub>2</sub> atmosphere for 45 min at room temperature and pressure. The reaction mixture was then filtered through celite and the solvent was removed under vacuum. **48** was obtained

as a yellow-brown amorphous solid (14.5 mg, 0.0529 mmol, 80%).

**mp** (MeOH)  $T / ^\circ\text{C} = 176$

**IR** (neat)  $\nu_{\text{max}} / \text{cm}^{-1} = 30000$  (O-H, br) 29251 (C-H), 28549 (C-H), 16133 (C=O)

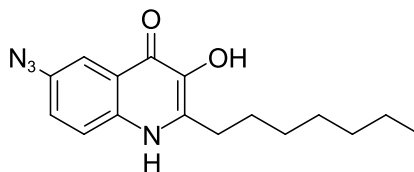
**$^1\text{H}$  NMR** (400 MHz, MeOD)  $\delta / \text{ppm} = 11.12$  (s, 1 H,  $\text{NH}$ ), 7.47 (d,  $J = 8.9$  Hz, 1 H, *meta* to C=O), 7.40 (d,  $J = 2.4$  Hz, 1 H, *ortho* to C=O), 7.16 (dd,  $J = 2.6, 9.0$  Hz, 1 H, *para* to C=O), 2.86 (t,  $J = 7.5$  Hz, 2 H,  $\text{CCH}_2$ ), 1.75 (quin,  $J = 7.8$  Hz, 2 H,  $\text{CCH}_2\text{CH}_2$ ), 1.48 - 1.22 (m,  $J = 5.4$  Hz, 8 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.89 (t,  $J = 6.7$  Hz, 3 H,  $\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz, MeOD)  $\delta / \text{ppm} = 166.8$  ( $\text{C}(=\text{O})$ ), 144.8 (*para* to  $\text{NH}_2$  or *ipso* to  $\text{C}(=\text{O})$ ), 140.5 ( $\text{COH}$ ), 138.6 ( $\text{C}=\text{COH}$ ), 132.6 (*ipso* to  $\text{NH}_2$ ), 124.8 (*para* to  $\text{NH}_2$  or *ipso* to  $\text{C}(=\text{O})$ ), 123.8 (*para* to  $\text{C}(=\text{O})$ ), 107.7 (*meta* to  $\text{NH}_2$  and *meta* to  $\text{C}(=\text{O})$ ), 106.4 (*ortho* to  $\text{NH}_2$  and *ortho* to  $\text{C}(=\text{O})$ ), 33.0 ( $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 29.5 - 31.0 ( $\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 23.8 ( $\text{CH}_2\text{CH}_3$ ), 14.5 ( $\text{CH}_3$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z / \text{Da} = 275.1760$ ,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_2]^+$  requires 275.1762

Spectroscopic data are not consistent with the literature.<sup>164</sup> It is possible that Baker's product is a Zn adduct.

## 9.12 6-Azido-2-heptyl-3-hydroxyquinolin-4(1H)-one **49**



6-Amino-2-heptyl-3-hydroxyquinolin-4(1H)-one **48** (18.2 mg, 0.0664 mmol, 1 eq.) was dissolved in HCl (conc., aq., 0.8 mL) and MeOH (0.5 mL) at  $0^\circ\text{C}$ .  $\text{NaNO}_2$  (5.0 mg, 0.0725 mmol, 1.09 eq.) in water (0.2 mL) was added dropwise over 2 min and the mixture was stirred at  $0^\circ\text{C}$  for 50 min, during which time the solution turned from yellow to orange.  $\text{NaN}_3$  (4.9 mg, 0.0754 mmol, 1.14 eq.) in water (0.2 mL) was then added and the mixture was allowed to warm to r.t. and stirred for 4 h. The reaction mixture was then filtered and the solid was dried under reduced pressure. **49** was obtained as a brown amorphous solid (5.5 mg, 0.0183 mmol, 28%).

**IR** (neat)  $\nu_{\text{max}} / \text{cm}^{-1} = 3089$  (N-H), 2921 (C-H), 2851 (C-H), 2108 (azide), 1632 (C=O)

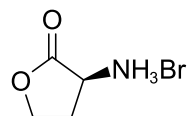
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO-d}_6$ )  $\delta / \text{ppm} = 7.74$  (s, 1 H, *ortho* to C=O), 7.65 (d,  $J = 6.9$  Hz, 1 H, *meta* to  $\text{C}(=\text{O})$ ), 7.32 (d,  $J = 7.4$  Hz, 1 H, *para* to  $\text{C}(=\text{O})$ ), 2.75 (t,  $J = 7.5$  Hz, 2 H,  $\text{CCH}_2$ ), 1.67 (quin,  $J = 6.4$  Hz, 2 H,  $\text{CCH}_2\text{CH}_2$ ), 1.43 - 1.13 (m, 8 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.85 (t,  $J = 6.8$  Hz, 3 H,  $\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{DMSO-d}_6$ )  $\delta / \text{ppm} = 166.3$  ( $\text{C}(=\text{O})$ ), 137.9 ( $\text{C}=\text{COH}$ ), 137.8 ( $\text{CN}_3$ ), 134.5 (*ipso* to  $\text{C}(=\text{O})$ ), 133.9 ( $\text{C}=\text{COH}$ ), 122.7 (*para* to  $\text{C}(=\text{O})$ ), 122.6 (*meta* to  $\text{N}_3$  and *meta* to  $\text{C}(=\text{O})$ ), 120.4 (*para* to  $\text{N}_3$ ), 112.4 (*ortho* to  $\text{N}_3$  and *ortho* to  $\text{C}(=\text{O})$ ), 31.2 ( $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 28.8 ( $\text{CCH}_2$ ), 28.4 ( $\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 28.3 ( $\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 27.8 ( $\text{CCH}_2\text{CH}_2$ ), 22.1 ( $\text{CH}_2\text{CH}_3$ ), 14.0 ( $\text{CH}_3$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z / \text{Da} = 301.1649$ ,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}_2]^+$  requires 301.1659

Spectroscopic data are consistent with the literature.<sup>164</sup>

### 9.13 (*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52**



L-Methionine **50** (3.04 g, 20.4 mmol, 1 eq.) and bromoacetic acid **51** (3.08 g, 22.2 mmol, 1.09 eq.) were dissolved in *i*-PrOH (12.5 mL), H<sub>2</sub>O (12.5 mL) and AcOH (5 mL). The reaction was refluxed for 15 h then concentrated under vacuum. The resulting brown oil was added to a mixture of *i*-PrOH (16 mL) and HBr (33% in AcOH, 4 mL), causing the precipitation of a pale pink amorphous solid. The precipitate was collected by filtration and washed with *i*-PrOH (20 mL). The filtrate was concentrated under vacuum and precipitated again using the same procedure. The two crops of precipitate were combined. **52** was obtained as a pale pink amorphous solid (1.73 g, 9.50 mmol, 41% yield).

**mp**  $T / ^\circ\text{C} = 242$  (*i*-PrOH/AcOH, gas evolved)

**IR** (neat)  $\nu_{\text{max}} / \text{cm}^{-1} = 2972$  (N-H), 2878 (N-H), 1772 (C=O), 1585 (N-H bend), 1572 (N-H bend)

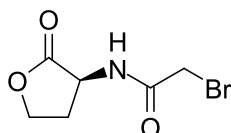
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta / \text{ppm} = 8.59$  (br s, 3 H,  $\text{NH}_3^+$ ), 4.46 (dt,  $J = 1.3, 8.9$  Hz, 1 H, OCHH), 4.37 (dd,  $J = 8.8, 11.4$  Hz, 1 H, CHNH<sub>3</sub><sup>+</sup>), 4.29 (ddd,  $J = 6.1, 8.8, 10.9$  Hz, 1 H, OCHH), 2.57 (dddd,  $J = 1.2, 6.1, 8.9, 12.3$  Hz, 1 H, OCH<sub>2</sub>CHH), 2.26 (dtd,  $J = 9.0, 11.2, 12.2$  Hz, 1 H, OCH<sub>2</sub>CHH)

**<sup>13</sup>C NMR** (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta / \text{ppm} = 173.3$  (C=O), 66.2 (OCH<sub>2</sub>), 47.8 (CHNH<sub>3</sub><sup>+</sup>), 27.0 (OCH<sub>2</sub>CH<sub>2</sub>)

$[\alpha]_D^{20} / ^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = -30.0$ , lit. =  $-25.0$  ( $c / \text{g}(100 \text{ mL})^{-1} = 0.0200$ , DMSO)

The data are consistent with the literature.<sup>165</sup>

### 9.14 (*S*)-2-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)acetamide **54**



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.08 eq.) and NaHCO<sub>3</sub> (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and water (2 mL). Bromoacetyl bromide **53** (44.0  $\mu\text{L}$ , 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 24 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The aqueous phase was extracted with EtOAc (4 $\times$ 10 mL). The combined organic layers were dried with MgSO<sub>4</sub> and the solvent was removed under reduced pressure. **54** was obtained as white, needle-like crystals (88.0 mg, 0.396 mmol, 74%).

**mp**  $T / ^\circ\text{C} = 132$  (EtOAc)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3256 (N-H), 3067 (C-H), 1763 (lactone C=O), 1658 (amide C=O), 1553 (N-H bend)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 6.94 (br s, 1 H,  $\text{NH}$ ), 4.57 (ddd,  $J$  = 11.7, 8.6, 5.9 Hz, 1 H,  $\text{CHNH}$ ), 4.51 (td,  $J$  = 9.2, 1.0 Hz, 1 H,  $\text{OCHH}$ ), 4.32 (ddd,  $J$  = 11.3, 9.4, 5.9 Hz, 1 H,  $\text{OCHH}$ ), 3.93 (s, 1 H,  $\text{CHHBr}$ ), 3.93 (s, 1 H,  $\text{CHHBr}$ ), 2.87 (dddd,  $J$  = 12.6, 8.6, 5.9, 1.3 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.22 (dtd,  $J$  = 12.6, 11.5, 11.5, 8.9 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ )

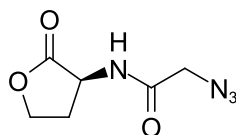
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 174.6 ( $\text{OC=O}$ ), 166.4 ( $\text{C(=O)NH}$ ), 66.1 ( $\text{OCH}_2$ ), 49.8 ( $\text{CHNHC=O}$ ), 29.9 ( $\text{OCH}_2\text{CH}_2$ ), 28.2 ( $\text{O=CCH}_2\text{Br}$ )

**HRMS** The compound does not ionise.

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = 27.0, lit. = 20.5 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.00740,  $\text{CHCl}_3$ )

The data are consistent with the literature.<sup>165,239</sup>

### 9.15 (*S*)-2-Azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide **55**



(3*S*)-2-Oxotetrahydrofuran-3-aminium bromide **52** (100 mg, 0.552 mmol, 1.08 eq.),  $\text{NaN}_3$  (85.7 mg, 1.32 mmol, 2.61 eq.) and  $\text{NaHCO}_3$  (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and water (2 mL). Bromoacetyl bromide **53** (44.0  $\mu\text{L}$ , 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the  $\text{CH}_2\text{Cl}_2$  was removed under vacuum. The aqueous phase was extracted with EtOAc (4 $\times$ 10 mL). The combined organic layers were dried with  $\text{MgSO}_4$  and the solvent was removed under reduced pressure. **55** was obtained as white, needle-like crystals (38.4 mg, 0.209 mmol, 41%).

**mp**  $T$  /  $^{\circ}\text{C}$  = 87 (EtOAc)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3284 (N-H), 2923 (C-H), 2853 (C-H), 2130 ( $\text{N}_3$ ), 1783 (lactone C=O), 1661 (amide C=O), 1537 (N-H bend)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 7.05 (br d,  $J$  = 6.5 Hz, 1 H,  $\text{NH}$ ), 4.64 (ddd,  $J$  = 11.6, 8.7, 6.8 Hz, 1 H,  $\text{CHNH}$ ), 4.48 (td,  $J$  = 9.1, 1.3 Hz, 1 H,  $\text{OCHH}$ ), 4.30 (ddd,  $J$  = 11.2, 9.2, 6.0 Hz, 1 H,  $\text{OCHH}$ ), 4.04 (s, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.76 (dddd,  $J$  = 12.5, 8.8, 6.0, 1.4 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.25 (dtd,  $J$  = 12.5, 11.4, 11.4, 8.9 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ )

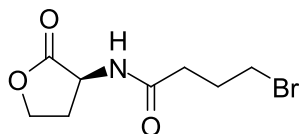
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 174.9 ( $\text{OC=O}$ ), 167.5 ( $\text{C=ONH}$ ), 66.0 ( $\text{OCH}_2$ ), 52.2 ( $\text{O=CCH}_2\text{N}_3$ ), 48.9 ( $\text{CHNHC=O}$ ), 29.7 ( $\text{OCH}_2\text{CH}_2$ )

**HRMS** The compound does not ionise.

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = -32.6, lit. = -24.4 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.0430, DMSO)

The data are consistent with the literature.<sup>165</sup>

### 9.16 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **57**



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (200 mg, 1.10 mmol, 1.00 eq.) and NaHCO<sub>3</sub> (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and water (2 mL). Bromobutyryl chloride **56** (140  $\mu$ L, 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The aqueous phase was extracted with EtOAc (7 $\times$ 5 mL) and the combined organic layers were dried with MgSO<sub>4</sub>. The solvent was removed under vacuum to give white crystals which were recrystallised from EtOAc. **57** was obtained as white, needle-like crystals (219 mg, 0.878 mmol, 80%).

mp *T* / °C = 105 (EtOAc)

IR (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3308 (N-H), 3074 (C-H), 2949 (C-H), 1774 (lactone C=O), 1644 (amide C=O), 1541 (N-H bend)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.31 (br d, *J* = 5.5 Hz, 1 H, NH), 4.59 (ddd, *J* = 6.2, 8.7, 11.5 Hz, 1 H, CHNH), 4.48 (dt, *J* = 1.2, 8.9 Hz, 1 H, OCHH), 4.30 (ddd, *J* = 5.8, 9.3, 11.3 Hz, 1 H, OCHH), 3.49 (t, *J* = 6.3 Hz, 2 H, CH<sub>2</sub>Br), 2.82 (dddd, *J* = 1.3, 5.9, 8.7, 12.5 Hz, 1 H, OCH<sub>2</sub>CHH), 2.47 (t, *J* = 7.3 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.26 - 2.15 (m, 3 H, OCH<sub>2</sub>CHH and CH<sub>2</sub>CH<sub>2</sub>Br)

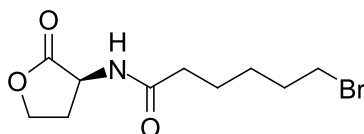
<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 175.4 (OC=O), 172.3 (C(=O)NH), 66.1 (OCH<sub>2</sub>), 49.3 (CHNHC=O), 33.9 (C(=O)CH<sub>2</sub>), 33.1 (CH<sub>2</sub>Br), 30.3 (OCH<sub>2</sub>CH<sub>2</sub>), 27.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

HRMS The compound does not ionise.

$[\alpha]_D^{26.6}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -78 (*c* / g(100 mL)<sup>-1</sup> = 0.0833, MeOH)

The compound has not been reported previously.

### 9.17 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **60**



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.00 eq.) and NaHCO<sub>3</sub> (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and water (2 mL) at r.t.. Bromohexanoyl chloride **59** (93.0  $\mu$ L, 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The mixture was then filtered, washed with water (10 mL) and dried under high vacuum. **60** was obtained as white, needle-like crystals (101 mg, 0.362 mmol, 66%).



**mp**  $T / ^\circ\text{C} = 106$  ( $\text{CH}_2\text{Cl}_2$ , water)

**IR** (neat)  $\nu_{\text{max}} / \text{cm}^{-1} = 3300$  (N-H), 3068 (C-H), 2937 (C-H), 2857 (C-H), 1785 (lactone C=O), 1639 (amide C=O), 1540 (N-H bend)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 6.09$  (br d,  $J = 5.7$  Hz, 1 H,  $\text{NH}$ ), 4.57 (ddd,  $J = 5.9, 8.6, 11.6$  Hz, 1 H,  $\text{CHNH}$ ), 4.50 (dt,  $J = 1.3, 9.1$  Hz, 1 H,  $\text{OCHH}$ ), 4.31 (ddd,  $J = 5.9, 9.3, 11.3$  Hz, 1 H,  $\text{OCHH}$ ), 3.43 (t,  $J = 6.7$  Hz, 2 H,  $\text{CH}_2\text{Br}$ ), 2.88 (dddd,  $J = 1.3, 5.9, 8.6, 12.6$  Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.30 (dt,  $J = 1.8, 7.5$  Hz, 2 H,  $\text{C(=O)CH}_2$ ), 2.16 (dtd,  $J = 8.9, 11.5, 12.5$  Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 1.90 (quin,  $J = 7.2$  Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{Br}$ ), 1.71 (quin,  $J = 7.6$  Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ ), 1.59 - 1.46 (m, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2$ )

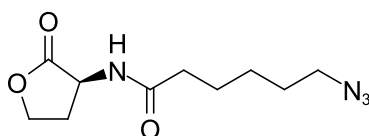
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 175.5$  ( $\text{OC=O}$ ), 173.3 ( $\text{C(=O)NH}$ ), 66.1 ( $\text{OCH}_2$ ), 49.3 ( $\text{CHNHC=O}$ ), 35.8 ( $\text{CH}_2\text{Br}$ ), 33.5 ( $\text{C(=O)CH}_2$ ), 32.3 ( $\text{CH}_2\text{CH}_2\text{Br}$ ), 30.5 ( $\text{OCH}_2\text{CH}_2$ ), 27.6 ( $\text{C(=O)CH}_2\text{CH}_2$ ), 24.4 ( $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z / \text{Da} = 278.0381$ ,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{10}\text{H}_{17}\text{BrNO}_3]^+$  requires 278.0386

$[\alpha]_D^{26.6} / ^\circ 10^{-1}\text{cm}^2\text{g}^{-1} = -16$  ( $c / \text{g}(100 \text{ mL})^{-1} = 0.208$ , MeOH)

The compound has not been reported previously.

## 9.18 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61**



(*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **60** (80 mg, 0.320 mmol, 1.00 eq.) and  $\text{NaN}_3$  (26.3 mg, 0.405 mmol, 1.27 eq.) were heated in DMF (0.5 mL) for 5 h at  $100^\circ\text{C}$ . The reaction mixture was then partitioned between  $\text{CH}_2\text{Cl}_2$  (5 mL) and water (5 mL). The aqueous phase was extracted twice more with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 5$  mL) and the organic layers were combined and dried over  $\text{MgSO}_4$ . The solvent was removed by rotary evaporation followed by high vacuum. **61** was obtained as white, needle-like crystals (42.7 mg, 0.178 mmol, 56%).

**mp**  $T / ^\circ\text{C} = 90$  ( $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\text{max}} / \text{cm}^{-1} = 3314$  (N-H), 2932 (C-H), 2863 (C-H), 2095 ( $\text{N}_3$ ), 1775 (lactone C=O), 1643 (amide C=O), 1548 (N-H bend)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 5.96$  (d,  $J = 4.2$  Hz, 1 H,  $\text{NH}$ ), 4.54 (ddd,  $J = 11.7, 8.6, 5.7$  Hz, 1 H,  $\text{CHNH}$ ), 4.49 (td,  $J = 9.1, 1.0$  Hz, 1 H,  $\text{OCHH}$ ), 4.30 (ddd,  $J = 11.3, 9.4, 5.8$  Hz, 1 H,  $\text{OCHH}$ ), 3.29 (t,  $J = 6.9$  Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.88 (dddd,  $J = 12.5, 8.6, 5.8, 1.1$  Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.28 (t,  $J = 7.5$  Hz, 1 H,  $\text{C(=O)CHH}$ ), 2.28 (t,  $J = 7.4$  Hz, 1 H,  $\text{C(=O)CHH}$ ), 2.14 (dtd,  $J = 12.3, 11.5, 11.5, 8.8$  Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 1.70 (quin,  $J = 7.6$  Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{N}_3$ ), 1.63 (quin,  $J = 7.2$  Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ ), 1.38 - 1.49 (m, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2$ )

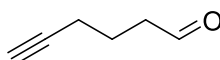
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 175.4 ( $\text{OC}=\text{O}$ ), 172.2 ( $\text{C}(=\text{O})\text{NH}$ ), 66.1 ( $\text{OCH}_2$ ), 51.2 ( $\text{CH}_2\text{N}_3$ ), 49.4 ( $\text{CHNHC}=\text{O}$ ), 35.9 ( $\text{C}(=\text{O})\text{CH}_2$ ), 30.7 ( $\text{OCH}_2\text{CH}_2$ ), 28.6 ( $\text{CH}_2\text{CH}_2\text{N}_3$ ), 26.3 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 24.8 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2$ )

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 241.1289,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{10}\text{H}_{17}\text{N}_4\text{O}_3]^+$  requires 241.1295

$[\alpha]_D^{26.6}$  /  $^{\circ}\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -16 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.208, MeOH)

The compound has not been reported previously.

## 9.19 Hex-5-ynal **63**



Pyridinium chlorochromate (14.6 g, 68.1 mmol, 1.50 eq) and  $\text{CH}_2\text{Cl}_2$  (500 mL) were stirred at r.t. under argon. 5-Hexyn-1-ol **62** (5.00 mL, 45.4 mmol, 1 eq.) was added and the reaction mixture was stirred for 5 h followed by addition of  $\text{Et}_2\text{O}$  (125 mL) and silica gel (62.5 g). The suspension was stirred for 1 h then filtered through a pad of silica (100 g) and washed with  $\text{Et}_2\text{O}$ . The solvent was removed by rotary evaporation. **63** was obtained as a pale yellow-green oil (4.72 g, 49.1 mmol, 72%).

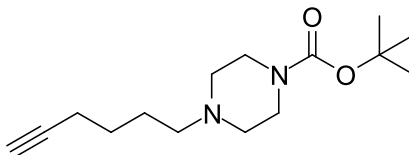
IR (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 3293 (alkyne C-H), 2943 (alkane C-H), 2831 (aldehyde C-H), 2729 (aldehyde C-H), 1720 (aldehyde C=O)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 9.80 (s, 1 H,  $\text{C}(=\text{O})\text{H}$ ), 2.60 (t,  $J$  = 7.1 Hz, 2 H,  $\text{CH}_2\text{C}(=\text{O})\text{H}$ ), 2.26 (dt,  $J$  = 2.6, 6.8 Hz, 2 H,  $\text{HC}\equiv\text{CCH}_2$ ), 1.98 (t,  $J$  = 2.7 Hz, 1 H,  $\text{HC}\equiv\text{C}$ ), 1.85 (quin,  $J$  = 7.0 Hz, 2 H,  $\text{HC}\equiv\text{CCH}_2\text{CH}_2$ )

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 201.6 ( $\text{C}(=\text{O})$ ), 83.1 ( $\text{HC}\equiv\text{C}$ ), 69.3 ( $\text{HC}\equiv\text{C}$ ), 42.4 ( $\text{CH}_2\text{C}(=\text{O})$ ), 20.7 ( $\text{CH}_2\text{CH}_2\text{C}(=\text{O})$ ), 17.6 ( $\text{HC}\equiv\text{CCH}_2$ )

Spectroscopic data are consistent with the literature.<sup>176</sup>

## 9.20 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate **65**



Hex-5-ynal **63** (0.407 g, 4.24 mmol, 1.00 eq.) and *tert*-butyl piperazine-1-carboxylate **64** (0.791 g, 4.24 mmol, 1.00 eq.) were stirred under a  $\text{N}_2$  atmosphere in 1,2-dichloroethane (20 mL) for 2.5 h followed by addition of sodium triacetoxyborohydride (6.25 g, 29.5 mmol, 7 eq.) in four portions over 4 d. The mixture was stirred for a further day then  $\text{NaHCO}_3$  (sat., aq., 120 mL) was added and the product extracted with  $\text{EtOAc}$  ( $2 \times 100$  mL). The solvent was dried over  $\text{MgSO}_4$  and removed by rotary evaporation. **65** was obtained as a colourless liquid (1.12 g, 4.21 mmol, 99%).

**TLC**  $R_f$  (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) = 0.55

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3304 (alkyne C-H), 2940 (alkane C-H), 2865 (C-H), 2810 (C-H), 1691 (carbamate C=O)

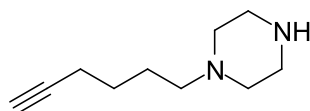
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 3.44 (t,  $J$  = 5.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (t,  $J$  = 5.1 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.37 (t,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.23 (dt,  $J$  = 2.7, 6.8 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.96 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.53 (m, 4 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.47 (s, 9 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 154.7 (NC(=O)O), 84.2 (HC≡C), 79.6 (C(CH<sub>3</sub>)<sub>3</sub>), 68.5 (HC≡C), 60.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 58.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 53.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 26.3 (CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 18.3 (HC≡CCH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 267.2073, [M+H]<sup>+</sup>, [C<sub>15</sub>H<sub>27</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup> requires 267.2064

The compound has not been reported previously.

## 9.21 1-(Hex-5-yn-1-yl)piperazine **66**



*tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate **65** (763 mg, 2.86 mmol) was stirred in TFA (10 mL) at r.t. for 2 h. The TFA was removed under vacuum followed by co-evaporation with CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL). The oil was diluted with water (10 mL) and the pH adjusted to 14 with NaOH (10% aq.). This mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL) and the combined organic layers were dried over MgSO<sub>4</sub>. The solvent was removed under vacuum and purified by column chromatography (SiO<sub>2</sub> MeOH/CH<sub>2</sub>Cl<sub>2</sub> 3:7). **66** was obtained as a colourless liquid (476 mg, 2.86 mmol, >99%).

**TLC**  $R_f$  (30% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) = 0.20

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3296 (alkyne C-H), 2941 (alkane C-H), 2811 (alkane C-H), 1637 (N-H bend)

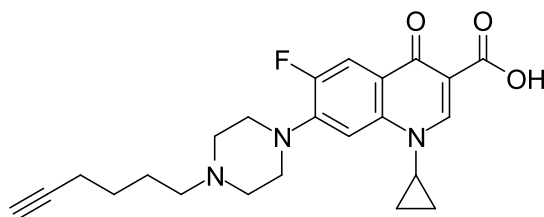
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 2.88 (t,  $J$  = 4.9 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.31 (t,  $J$  = 7.1 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.20 (dt,  $J$  = 2.7, 6.8 Hz, 2 H, HC≡CCH<sub>2</sub>), 2.05 (br s, 1 H, NH), 1.93 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.48 (m, 4 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 84.3 (HC≡C), 68.4 (HC≡C), 58.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 54.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 46.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 26.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 18.3 (HC≡CCH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 167.1548, [M+H]<sup>+</sup>, [C<sub>10</sub>H<sub>19</sub>N<sub>2</sub>]<sup>+</sup> requires 167.1548

The compound has not been reported previously.

## 9.22 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68**



7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **67** (1.27 g, 4.51 mmol, 1 eq.), 1-(hex-5-yn-1-yl)piperazine **66** (1.5 g, 9.02 mmol, 2 eq.) and *N*-methyl-2-pyrrolidone (10 mL) were stirred in a microwave reactor at 115 °C for 24 h. The reaction mixture was cooled to r.t. and water (80 mL) was added. The mixture was stirred for 3 h and then filtered, and residue was washed with MeOH (50 mL). The resulting solid (0.571 g) was further purified by recrystallisation from EtOAc (50 mL). **68** was obtained as off-white crystals (0.219 g, 0.531 mmol, 12%).

**TLC**  $R_f$  = 0.02 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**mp**  $T$  / °C = 220 (MeOH, decomposes)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3212 (alkyne C-H), 2459 (O-H), 1723 (carboxylic acid C=O), 1627 (quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 15.12 (br s, 1 H, C(=O)OH), 8.69 (s, 1 H, *ortho* to C(=O)OH), 7.96 (d,  $J$  = 13.0 Hz, 1 H, *ortho* to F), 7.61 (d,  $J$  = 7.6 Hz, 1 H, *meta* to F), 3.82 - 3.92 (m, 3 H, NCH(CH<sub>2</sub>)<sub>2</sub> and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.54 - 3.68 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 3.45 (br. t,  $J$  = 11.6 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.21 - 3.29 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.11 - 3.20 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.84 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 2.24 (td,  $J$  = 7.0, 2.7 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.83 (br. quin,  $J$  = 7.5 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin,  $J$  = 7.4 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.36 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.16 - 1.23 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

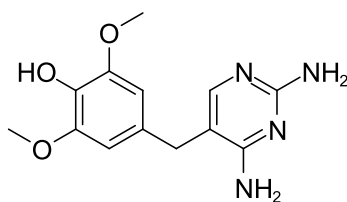
**<sup>13</sup>C NMR** (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 165.8 (C(=O)OH), 152.8 (d,  $J$  = 248.5 Hz, *ipso* to F), 148.2 (CHCC(=O)OH), 143.7 (d,  $J$  = 11.1 Hz, *para* to C(=O)), 139.1 (*para* to F), 119.4 (d,  $J$  = 6.9 Hz, *ipso* to C(=O)), 111.2 (d,  $J$  = 22.5 Hz, *ortho* to F and *ortho* to C(=O)), 106.9 (*meta* to F and *meta* to C(=O)), 106.9 (C(=O)CC(=O)OH), 83.9 (HC≡C), 71.8 (HC≡C), 55.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 50.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 46.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.0 (NCH(CH<sub>2</sub>)<sub>2</sub>), 25.2 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 22.3 (HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 17.4 (HC≡CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -121.8 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 412.2036, [M+H]<sup>+</sup>, [C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>F]<sup>+</sup> requires 412.2030

The compound has not been reported previously.

### 9.23 4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol **69**



Hydrobromic acid (48% w/w, aq., 50 mL) was heated to 100 °C. Trimethoprim **25** (5.00 g, 17.2 mmol) was added, and the suspension was stirred for 40 min under Ar. The mixture was removed from the heat, and NaOH (50% w/w, aq., 15 mL) was added dropwise. The reaction mixture was then cooled slowly to 0 °C, and the resulting crystals were filtered out and washed with cold water. The crystals were then dissolved in hot water (80 mL), neutralized with NH<sub>4</sub>OH (sat., aq.) and cooled slowly to 0 °C. The resulting crystals were filtered out, washed with cold water and dried under vacuum. **69** was obtained as pale pink prisms (2.06 g, 7.46 mmol, 43%).

**TLC**  $R_f$  = 0.04 (5% MeOH/CHCl<sub>2</sub>)

**mp**  $T$  / °C = 238 (water, decomposes)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3314 (N-H), 3137 (N-H), 3045 (C-H), 3001 (C-H), 2938 (C-H), 2839 (C-H), 1663 (pyrimidine), 1645 (pyrimidine), 1627 (pyrimidine)

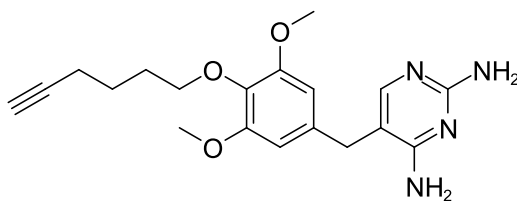
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 7.21 (s, 1 H, CHN), 6.54 (s, 2 H, *meta* to OCH<sub>3</sub>), 4.87 (br s, 5 H, OH, NH<sub>2</sub> × 2), 3.82 (s, 6 H, OCH3), 3.63 (s, 2 H, CCH2C)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 166.4 (CH<sub>2</sub>CCNH<sub>2</sub>), 162.0 (CHNCNH<sub>2</sub>), 156.2 (CHNCNH<sub>2</sub>), 149.8 (*ipso* to OCH<sub>3</sub>), 135.9 (*ipso* to OH), 128.2 (*para* to OH), 111.7 (CH<sub>2</sub>CCNH<sub>2</sub>), 107.5 (*meta* to OH), 57.0 (OCH<sub>3</sub>), 33.9 (CCH<sub>2</sub>C)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 277.1295, [M+H]<sup>+</sup> found, [C<sub>13</sub>H<sub>17</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 277.1301

The data are consistent with the literature.<sup>167</sup>

### 9.24 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71**



4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol **69** (1.00 g, 3.62 mmol, 1 eq.), 6-chloro-1-hexyne **70** (0.524 mL, 0.420 g, 4.34 mmol, 1.2 eq.), Cs<sub>2</sub>CO<sub>3</sub> (2.36 g, 7.24 mmol, 2 eq.) and anhydrous DMF (30 mL) were stirred at 70 °C for 7 h. The solvent was removed under reduced pressure, then CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was

added and the mixture filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). **71** was obtained as a pale cream amorphous solid (0.327 g, 0.917 mmol, 25%).

**TLC**  $R_f$  = 0.14 (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3451 (alkyne C-H), 3313 (N-H), 3137 (N-H), 3114 (N-H), 2944 (C-H), 2839 (C-H), 1635 (pyrimidine)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 7.77 (s, 1 H, CHN), 6.37 (s, 2 H, *meta* to OCH<sub>2</sub>), 4.83 (br s, 2 H, CHNCNH<sub>2</sub>), 4.63 (br s, 2 H, CH<sub>2</sub>CCNH<sub>2</sub>), 3.95 (t,  $J$  = 6.3 Hz, 2 H, CH<sub>2</sub>O), 3.79 (s, 6 H, OCH<sub>3</sub>), 3.65 (s, 2 H, CCH<sub>2</sub>C), 2.28 (td,  $J$  = 7.1, 2.6 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.94 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.81 - 1.90 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>O), 1.71 - 1.80 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 162.7 (CH<sub>2</sub>CCNH<sub>2</sub>), 162.0 (CHNCNH<sub>2</sub>), 156.4 (CHNCNH<sub>2</sub>), 153.8 (*ipso* to OCH<sub>3</sub>), 136.0 (*ipso* to OCH<sub>2</sub>), 133.6 (*para* to OCH<sub>2</sub>), 106.5 (CH<sub>2</sub>CCNH<sub>2</sub>), 105.0 (*meta* to OCH<sub>2</sub>), 84.5 (HC≡C), 72.6 (CH<sub>2</sub>O), 68.3 (HC≡C), 56.1 (OCH<sub>3</sub>), 34.7 (CCH<sub>2</sub>C), 29.1 (CH<sub>2</sub>CH<sub>2</sub>O), 24.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 18.0 (HC≡CCH<sub>2</sub>)

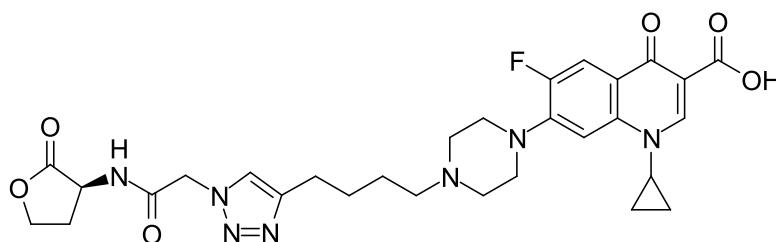
**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 357.1920, [M+H]<sup>+</sup> found, [C<sub>19</sub>H<sub>25</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 357.1927

The compound has not been reported previously.

## 9.25 Optimised general procedure for the click reaction

Azide (1 eq.) and alkyne (1 eq.) were dissolved in 50% *t*-BuOH/water in a round-bottomed flask with a stirrer bar, closed with a new septum. The mixture was degassed by bubbling through N<sub>2</sub>. The mixture was placed under positive pressure of Ar using a balloon. Equimolar amounts of CuSO<sub>4</sub> · 5H<sub>2</sub>O and THPTA **74** were dissolved in water to make a 50 mM solution and similarly degassed. Sodium ascorbate was dissolved in water to make a 100 mM solution and similarly degassed. The Cu/THPTA solution (0.05 eq.) was added to the reaction mixture, followed by the sodium ascorbate solution (0.1 eq.). The mixture was stirred for 2 h and monitored using LCMS. HL derivative conjugates were dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). Other conjugates were purified by preparative HPLC (5-95% acetonitrile/water over 20 min).

## 9.26 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **72**



50% water/*t*-BuOH (2 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-2-azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide **55** (9.2 mg, 50.0  $\mu$ mol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624  $\mu$ g, 2.5  $\mu$ mol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu$ mol, 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu$ g, 5  $\mu$ mol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (50  $\mu$ l) was then added. The mixture was stirred at r.t. under argon for 3 h. On observation that the reaction had stalled, the reaction was degassed again, and a further portion of catalyst solution (50  $\mu$ l) was added. After a further 3 h the reaction mixture was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **72** was obtained as a white amorphous solid (8.8 mg, 14.8  $\mu$ mol, 30%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3266 (N-H), 2949 (C-H), 2935 (C-H), 2827 (C-H), 1778 (lactone C=O), 1725 (carboxylic acid C=O), 1665 (amide C=O), 1626 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 15.23 (s, 1 H, C(=O)OH), 8.84 (d, *J* = 7.9 Hz, 1 H, NH), 8.66 (s, 1 H, *ortho* to C(=O)OH), 7.90 (d, *J* = 13.3 Hz, 1 H, *ortho* to F), 7.82 (s, 1 H, CH=CCH<sub>2</sub>), 7.57 (d, *J* = 7.6 Hz, 1 H, *meta* to F), 5.13 (s, 1 H, C(=O)CHHN), 5.12 (s, 1 H, C(=O)CHHN), 4.64 (ddd, *J* = 10.9, 9.0, 7.8 Hz, 1 H, CHNH), 4.36 (td, *J* = 8.9, 1.7 Hz, 1 H, OCHH), 4.23 (ddd, *J* = 10.6, 8.8, 6.4 Hz, 1 H, OCHH), 3.83 (tt, *J* = 7.0, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br s, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.67 (t, *J* = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 2.58 (br t, *J* = 5.0 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.42 - 2.49 (m, 1 H, OCH<sub>2</sub>CHH), 2.40 (t, *J* = 7.1 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.17 (dtd, *J* = 11.7, 10.8, 9.0 Hz, 1 H, OCH<sub>2</sub>CHH), 1.66 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.53 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.28 - 1.35 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.16 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

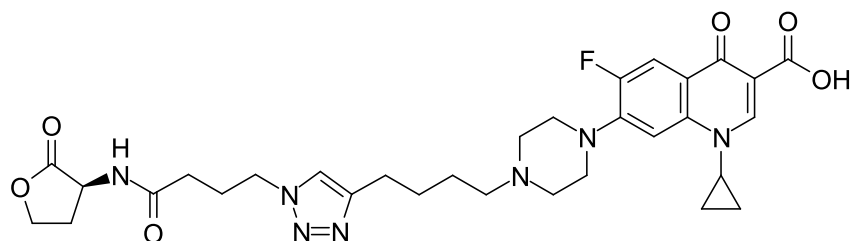
**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 174.9 (OC(=O)), 166.0 (C(=O)OH), 165.9 (NHC(=O)), 153.1 (d, *J* = 250.8 Hz, *ipso* to F), 148.0 (CH=CC(=O)OH), 146.6 (CH=CCH<sub>2</sub>), 145.3 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 123.4 (CH=CCH<sub>2</sub>), 118.5 (d, *J* = 7.5 Hz, *para* to piperazine), 110.9 (d, *J* = 23.5 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.4 (d, *J* = 3.2 Hz, *meta* to C=O and *meta* to F), 65.4 (OCH<sub>2</sub>), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.2 (C(=O)CH<sub>2</sub>N), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.2 (CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 28.2 (CH<sub>2</sub>CHNH), 26.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 596.2627, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>35</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 596.2633

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -3.5 (*c* / g(100 mL)<sup>-1</sup> = 0.0575, MeOH)

The compound has not been reported previously.

**9.27 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **77****



50% water/*t*-BuOH (2 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **58** (10.6 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (50 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **77** was obtained as a white amorphous solid (14.6 mg, 23.4 μmol, 47%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3287 (N-H), 2950 (C-H), 2821 (C-H), 2778 (C-H), 1778 (lactone C=O), 1726 (carboxylic acid C=O), 1664 (amide C=O), 1626 (quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 15.22 (br s, 1 H, C(=O)OH), 8.65 (s, 1 H, *ortho* to C(=O)OH), 8.40 (d, *J* = 8.0 Hz, 1 H, NH), 7.88 (d, *J* = 13.4 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH<sub>2</sub>), 7.55 (d, *J* = 7.5 Hz, 1 H, *meta* to F), 4.53 (ddd, *J* = 10.9, 9.0, 8.1 Hz, 1 H, CHNH), 4.33 (td, *J* = 8.9, 1.8 Hz, 1 H, OCHH), 4.31 (t, *J* = 7.0 Hz, 2 H, CH<sub>2</sub>NCH=C), 4.20 (ddd, *J* = 10.5, 8.8, 6.5 Hz, 1 H, OCHH), 3.82 (tt, *J* = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br. t, *J* = 4.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t, *J* = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br. t, *J* = 5.0 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 - 2.42 (m, 3 H, OCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.09 - 2.19 (m, 3 H, OCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>), 2.02 (quin, *J* = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.64 (quin, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.15 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.3 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 171.2 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 248.6 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 (CH=CCH<sub>2</sub>), 118.5 (d, *J* = 7.5 Hz, *para* to piperazine), 110.9 (d, *J* = 22.4 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.3 (d, *J* = 3.2 Hz, *meta* to C=O and *meta* to F), 65.3 (OCH<sub>2</sub>), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.6 (CH<sub>2</sub>NCH=C), 47.9 (OC(=O)CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 31.7 (NHC(=O)CH<sub>2</sub>), 28.2 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.8 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

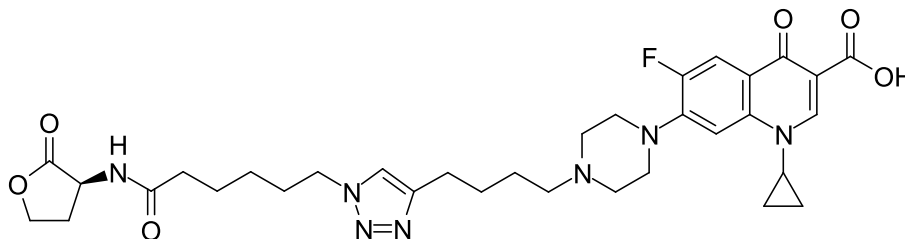
**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 624.2928, [M+H]<sup>+</sup> found, [C<sub>31</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 624.2946

[ $\alpha$ ]<sub>D</sub><sup>20</sup> / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -10.6 (*c* / g(100 mL)<sup>-1</sup> = 0.094, MeOH)



The compound has not been reported previously.

**9.28 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **78****



50% water/*t*-BuOH (2 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (12.0 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (50 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min) The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **78** was obtained as a white amorphous solid (12.4 mg, 19.0 μmol, 38%).

**TLC** *R<sub>f</sub>* = 0.30 (30% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3302 (N-H), 2940 (C-H), 2858 (C-H), 1785 (lactone C=O), 1729 (carboxylic acid C=O), 1658 (amide C=O), 1626 (quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 15.22 (br s, 1 H, C(=O)OH), 8.65 (s, 1 H, *ortho* to C(=O)OH), 8.32 (d, *J* = 8.0 Hz, 1 H, NH), 7.89 (d, *J* = 13.3 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H, CH=CCH<sub>2</sub>), 7.55 (d, *J* = 7.6 Hz, 1 H, *meta* to F), 4.51 (ddd, *J* = 10.9, 9.1, 7.9 Hz, 1 H, CHNH), 4.33 (td, *J* = 8.8, 1.8 Hz, 1 H, OCHH), 4.28 (t, *J* = 7.1 Hz, 2 H, CH<sub>2</sub>NCH=C), 4.19 (ddd, *J* = 10.5, 8.7, 6.6 Hz, 1 H, OCHH), 3.82 (tt, *J* = 7.0, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br t, *J* = 4.5, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.63 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br t, *J* = 4.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.33 - 2.41 (m, 3 H, OCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.06 - 2.16 (m, 3 H, OCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>), 1.79 (quin, *J* = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.63 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.45 - 1.56 (m, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.34 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.19 - 1.25 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.15 - 1.19 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 172.1 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 250.2 Hz, *ipso* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.6 (CH=CCH<sub>2</sub>), 118.5 (d, *J* = 8.0 Hz, *para* to piperazine), 110.9 (d, *J* = 23.5 Hz, *ortho* to C=O and *ortho* to F), 106.7 (C(=O)OH), 106.3 (d, *J* = 2.1 Hz, *meta* to C=O and *meta* to F), 65.3 (OCH<sub>2</sub>), 57.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.0 (CH<sub>2</sub>NCH=C), 47.8 (CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 34.8 (NHC(=O)CH<sub>2</sub>), 29.5 (CH<sub>2</sub>CH<sub>2</sub>NCH=C), 28.3 (CH<sub>2</sub>CHNH), 26.9 (CH=C

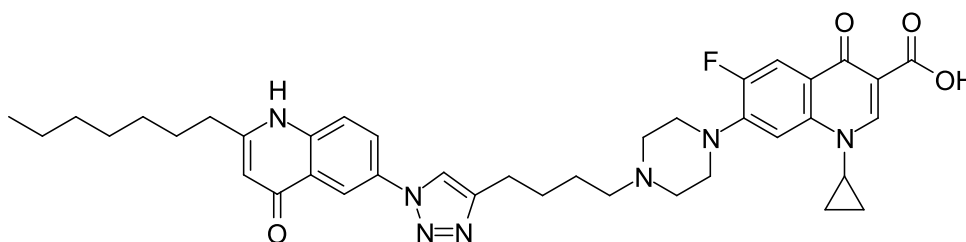
CH<sub>2</sub>CH<sub>2</sub>), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.4 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 24.5 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 652.3254, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 652.3248

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -8.5 ( $c$  / g(100 mL)<sup>-1</sup> = 0.106, MeOH)

The compound has not been reported previously.

**9.29 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1-*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **80****



50% water/*t*-BuOH (1 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (4.1 mg, 10.0 μmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1*H*)-one **38** (2.8 mg, 10.0 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5H<sub>2</sub>O (125 μg, 0.5 μmol, 0.05 eq. 50 mM), THPTA (218 μg, 0.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (198 μg, 1 μmol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (10 μl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then the reaction mixture was evaporated under reduced pressure. The residue was purified by preparative HPLC (50-100% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (10 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **80** was obtained as a white amorphous solid (8.6 mg, 2.7 μmol, 27%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2927 (C-H), 2866 (C-H), 1716 (carboxylic acid C=O), 1631 (ciprofloxacin quinolone C=O and HHQ C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) 15.12 (br s, 1 H, C(=O)OH), 11.79 (s, 1 H, NH), 8.75 (s, 1 H, NCH=CCH<sub>2</sub>), 8.71 (s, 1 H, *ortho* to C(=O)OH), 8.40 (d,  $J$  = 2.7 Hz, 1 H, *ortho* to C(=O) and *ortho* to N), 8.18 (dd,  $J$  = 8.9, 2.6 Hz, 1 H, *para* to C(=O) and *ortho* to N), 7.99 (d,  $J$  = 13.0 Hz, 1 H, *ortho* to F), 7.75 (d,  $J$  = 9.0 Hz, 1 H, *meta* to C(=O) and *meta* to N), 7.62 (d,  $J$  = 7.8 Hz, 1 H, *meta* to F), 6.02 (s, 1 H, NHC=CHC(=O)), 3.85 (tt,  $J$  = 7.0, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.23 - 3.30 (m, 10 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.82 (t,  $J$  = 5.9 Hz, 2 H, NCH=CCH<sub>2</sub>), 2.63 (t,  $J$  = 7.9 Hz, 2 H, CH<sub>2</sub>C=CHC(=O)), 1.76 - 1.81 (m, 4 H, NCH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.70 (quin,  $J$  = 7.2 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>C=CHC(=O)), 1.15 - 1.38 (m, 12 H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, NCH(CH<sub>2</sub>)<sub>2</sub> and NCH(CH<sub>2</sub>)<sub>2</sub>), 0.87 (t,  $J$  = 6.9 Hz, 3 H, CH<sub>3</sub>)

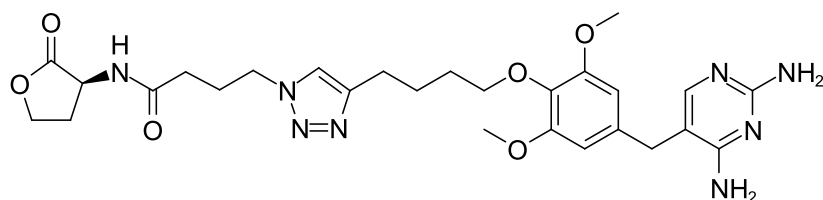
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 176.3 (CHC(=O)), 165.8 (C(=O)OH), 154.3 (CCHC(=O)), 152.9 (d,  $J$  = 240.1 Hz, *ipso* to F), 148.3 (CH=CC(=O)OH), 147.5 (NCHCCH<sub>2</sub>), 143.3 (d,  $J$  = 8.5 Hz, *ortho* to F and *ipso* to N), 139.6 (*ipso* to NH), 139.0 (*para* to F), 132.0 (*para* to NH), 124.9 (*ipso*

to C(=O) and *ortho* to NH), 123.6 (*para* to C(=O) and *meta* to NH), 120.5 (NCH=CCH<sub>2</sub>), 120.0 (*meta* to C(=O) and *meta* to N), 119.6 (d, *J* = 9.6 Hz, *ipso* to C(=O) and *para* to N), 115.1 (*ortho* to C(=O) and *ortho* to N), 111.3 (d, *J* = 28.8 Hz, *ortho* to F and *ortho* to C(=O)), 107.9 (*meta* to F and *meta* to C(=O)), 107.2 (CHC(=O)), 106.9 (CC(=O)OH), 55.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 50.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 46.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 46.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.0 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.2 (CH<sub>2</sub>CNH), 31.2 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.3 - 28.5 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 24.4 (CH=CCH<sub>2</sub>), 22.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 22.0 (CH<sub>3</sub>CH<sub>2</sub>), 13.9 (CH<sub>3</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 696.3667, [M+H]<sup>+</sup> found, [C<sub>39</sub>H<sub>47</sub>FN<sub>7</sub>O<sub>4</sub>]<sup>+</sup> requires 696.3668

The compound has not been reported previously.

### 9.30 (*S*)-4-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **84**



50% water/*t*-BuOH (2 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71** (20.6 mg, 50.0 μmol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **58** (15.9 mg, 75.0 μmol, 1.5 eq.). Similarly degassed solutions of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624 μg, 2.5 μmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (991 μg, 5 μmol, 0.1 eq., 100 mM) in water (50 μl) were then added. An extra portion of **58** (10.6 mg, 50.0 μmol, 1 eq.) was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks, the reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (6 × 10 mL) then dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **84** was obtained as a pale brown gum (4.8 mg, 8.4 μmol, 17%, purity 77% by NMR, contaminant was **58**).

**TLC** *R<sub>f</sub>* = 0.30 (30% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat) *ν<sub>max</sub>* / cm<sup>-1</sup> = 3341 (N-H), 3303 (N-H), 3183 (N-H), 2934 (C-H), 1774 (lactone C=O), 1660 (amide C=O and pyrimidine)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) *δ* / ppm = 8.43 (d, *J* = 8.0 Hz, 1 H, NH), 7.80 (s, 1 H, NCH=CCH<sub>2</sub>), 7.46 (s, 1 H, CHN=CNH<sub>2</sub>), 6.68 (br s, 2 H, CH<sub>2</sub>CCNH<sub>2</sub>), 6.53 (s, 2 H, *meta* to CH<sub>2</sub>), 6.21 (br s, 2 H, CHN=CNH<sub>2</sub>), 4.49 (dt, *J* = 10.7, 8.6 Hz, 1 H, CHNH), 4.32 (td, *J* = 8.7, 1.6 Hz, 1 H, CHHOC(=O)), 4.29 (t, *J* = 6.8 Hz, 2 H, CH<sub>2</sub>N), 4.19 (ddd, *J* = 10.6, 8.7, 6.5 Hz, 1 H, CHHOC(=O)), 3.79 (t, *J* = 6.2 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.68 (s, 6 H, CH<sub>3</sub>), 3.53 (br s, 2 H, CCH<sub>2</sub>C), 2.63 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.37 (dddd, *J* = 12.2, 8.9, 6.7, 1.8 Hz, 1 H, CHHCHNH), 2.08 - 2.15 (m, 3 H, CHHCHNH and C(=O)CH<sub>2</sub>), 2.00 (quin, *J* = 7.2 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N), 1.72 (quin, *J* = 7.3 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.61 (quin, *J* = 6.7 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>O)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>) *δ* / ppm = 175.8 (OC=O), 171.9 (NHC=O), 163.1 (CC(NH<sub>2</sub>)N), 159.7

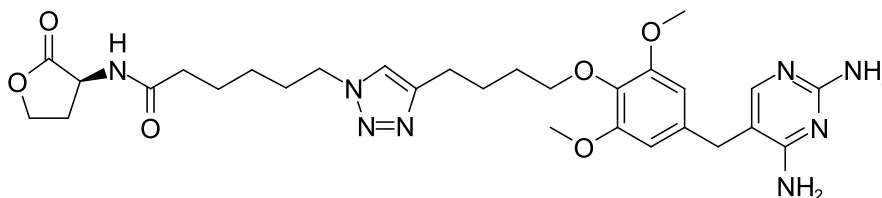
(br s,  $\text{NC}(\text{NH}_2)\text{N}$ ), 153.2 (*ipso* to  $\text{OCH}_3$ ), 150.5 (br s,  $\text{CHNC}(\text{NH}_2)\text{N}$ ), 147.3 ( $\text{NCH}=\text{CCH}_2\text{CH}_2$ ), 135.2 (*para* to  $\text{CH}_2\text{O}$ ), 135.0 (*ipso* to  $\text{CH}_2\text{O}$ ), 122.1 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 107.3 ( $\text{CH}_2\text{CC}(\text{NH}_2)=\text{N}$ ), 106.2 (*meta* to  $\text{CH}_2\text{O}$ ), 72.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 65.7 ( $\text{OCH}_2\text{CH}_2\text{CHNH}$ ), 56.2 ( $\text{OCH}_3$ ), 48.9 ( $\text{CH}_2\text{N}$ ), 48.3 ( $\text{CHNH}$ ), 32.9 ( $\text{CCH}_2\text{C}$ ), 32.0 ( $\text{C}(=\text{O})\text{CH}_2$ ), 29.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 28.4 ( $\text{OCH}_2\text{CH}_2\text{CHNH}$ ), 26.0 ( $\text{CH}_2\text{CH}_2\text{N}$ ), 25.7 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 24.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 569.2834,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{27}\text{H}_{37}\text{N}_8\text{O}_6]^+$  requires 569.2836

$[\alpha]_D^{20}$  /  $^\circ 10^{-1}\text{cm}^2\text{g}^{-1}$  = -4.6 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.0433, MeOH)

The compound has not been reported previously.

### 9.31 (*S*)-6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **85**



50% water/*t*-BuOH (2 mL) was degassed by bubbling  $\text{N}_2$  through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71** (20.6 mg, 50.0  $\mu\text{mol}$ , 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (18.0 mg, 75.0  $\mu\text{mol}$ , 1.5 eq.). Similarly degassed solutions of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (624  $\mu\text{g}$ , 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu\text{g}$ , 5  $\mu\text{mol}$ , 0.1 eq., 100 mM) in water (50  $\mu\text{l}$ ) were then added. An extra portion of **61** (12.0 mg, 50.0  $\mu\text{mol}$ , 1 eq.) was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks the reaction mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (6 $\times$ 10 mL) then dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography using a Combiflash ( $\text{SiO}_2$ , 0-20% MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **85** was obtained as a clear gum (8.0 mg, 13.4  $\mu\text{mol}$ , 27%).

**TLC**  $R_f$  = 0.35 (30% MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 3336 (N-H), 3209 (N-H), 2941 (C-H), 2869 (C-H), 1775 (lactone C=O), 1657 (amide C=O and pyrimidine)

**$^1\text{H}$  NMR** (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 8.34 (d,  $J$  = 8.0 Hz, 1 H,  $\text{NH}$ ), 7.83 (s, 1 H,  $\text{NCH}=\text{CCH}_2$ ), 7.50 (s, 1 H,  $\text{CHN}=\text{CNH}_2$ ), 6.54 (s, 2 H, *meta* to  $\text{CH}_2$ ), 6.17 (br s, 2 H,  $\text{CH}_2\text{CCNH}_2$ ), 5.77 (br s, 2 H,  $\text{CHN}=\text{CNH}_2$ ), 4.51 (ddd,  $J$  = 11.0, 9.0, 8.1 Hz, 1 H,  $\text{CHNH}$ ), 4.33 (td,  $J$  = 8.8, 1.9 Hz, 1 H,  $\text{CHHOC}(=\text{O})$ ), 4.27 (t,  $J$  = 7.1 Hz, 2 H,  $\text{CH}_2\text{N}$ ), 4.19 (ddd,  $J$  = 10.5, 8.7, 6.5 Hz, 1 H,  $\text{CHHOC}(=\text{O})$ ), 3.80 (t,  $J$  = 6.3 Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 3.70 (s, 6 H,  $\text{CH}_3$ ), 3.52 (s, 2 H,  $\text{CCH}_2\text{C}$ ), 2.64 (t,  $J$  = 7.5 Hz, 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.36 (dddd,  $J$  = 12.1, 8.9, 6.7, 1.8 Hz, 1 H,  $\text{CHHCHNH}$ ), 2.06 - 2.16 (m, 3 H,  $\text{CHHCHNH}$  and  $\text{C}(=\text{O})\text{CH}_2$ ), 1.78 (quin,  $J$  = 7.4 Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 1.73 (quin,  $J$  = 7.7 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 1.63 (quin,  $J$  = 6.8 Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{O}$ ), 1.52 (quin,  $J$  = 7.5 Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 1.17 - 1.27 (m, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2$ )

**$^{13}\text{C}$  NMR** (125 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 175.4 ( $\text{OC}=\text{O}$ ), 172.0 ( $\text{NHC}=\text{O}$ ), 162.2 ( $\text{CC}(\text{NH}_2)\text{N}$ ), 161.8

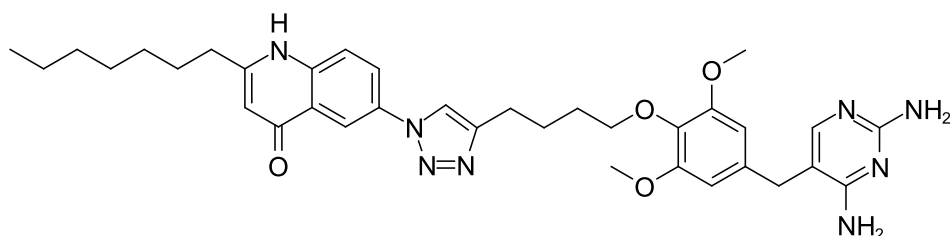
(NC(NH<sub>2</sub>)N), 154.8 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 135.5 (*para* to CH<sub>2</sub>O), 134.8 (*ipso* to CH<sub>2</sub>O), 121.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 105.9 (CH<sub>2</sub>CC(NH<sub>2</sub>)=N), 105.8 (*meta* to CH<sub>2</sub>O), 71.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.2 (OCH<sub>2</sub>CH<sub>2</sub>CHNH), 55.8 (OCH<sub>3</sub>), 49.0 (CH<sub>2</sub>N), 47.8 (CHNH), 34.8 (C(=O)CH<sub>2</sub>), 32.9 (CCH<sub>2</sub>C), 29.4 (CH<sub>2</sub>CH<sub>2</sub>N), 29.1 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 28.2 (OCH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 24.4 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 597.3149, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>41</sub>N<sub>8</sub>O<sub>6</sub>]<sup>+</sup> requires 597.3144

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -3.6 ( $c$  / g(100 mL)<sup>-1</sup> = 0.11, MeOH)

The compound has not been reported previously.

### 9.32 6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(1*H*)-one **87**



50% water/*t*-BuOH (1 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71** (3.6 mg, 10.0 μmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1*H*)-one **38** (2.8 mg, 10.0 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (125 μg, 0.5 μmol, 0.05 eq. 50 mM), THPTA (218 μg, 0.5 μmol, 0.05 eq. 50 mM) and sodium ascorbate (198 μg, 1 μmol, 0.1 eq., 100 mM) in water (10 μl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then evaporated under reduced pressure. The residue was purified by preparative HPLC (5-100% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (10 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **87** was obtained as a clear gum (2.6 mg, 4.1 μmol, 41%).

**TLC**  $R_f$  = 0.17 (20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2928 (C-H), 2856 (C-H), 1664 (pyrimidine), 1645 (pyrimidine and HHQ C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 11.80 (s, 1 H, NH), 8.69 (s, 1 H, NCH=CCH<sub>2</sub>), 8.41 (d,  $J$  = 2.7 Hz, 1 H, *ortho* to C=O), 8.17 (dd,  $J$  = 9.0, 2.6 Hz, 1 H, *para* to C=O), 7.73 (d,  $J$  = 9.0 Hz, 1 H, *ortho* to NH), 7.51 (br s, 4 H, NH<sub>2</sub>), 7.41 (s, 1 H, CHN=CNH<sub>2</sub>), 6.61 (s, 2 H, *meta* to CH<sub>2</sub>), 6.02 (d,  $J$  = 1.8 Hz, 1 H, C(=O)CH), 3.86 (t,  $J$  = 6.3 Hz, 2 H, CH<sub>2</sub>O), 3.73 (s, 6 H, OCH<sub>3</sub>), 3.57 - 3.62 (m, 2 H, CCH<sub>2</sub>C), 2.78 (t,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.63 (t,  $J$  = 7.3 Hz, 2 H, HNCCH<sub>2</sub>), 1.85 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.61 - 1.78 (m, 4 H, HNCCH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.31 - 1.40 (m, 4 H, HNCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.25 - 1.31 (m, 4 H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 0.86 (t,  $J$  = 7.2 Hz, 3 H, CH<sub>3</sub>CH<sub>2</sub>)

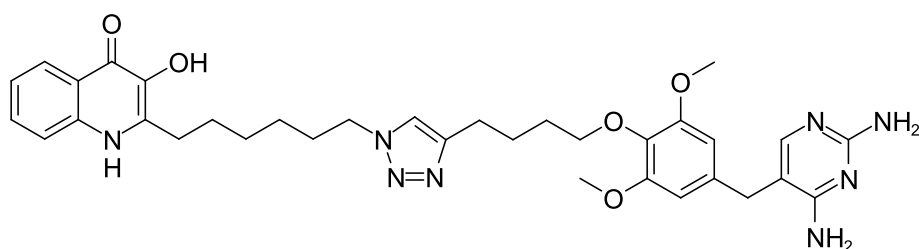
**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C=O), 164.1 (CC(NH<sub>2</sub>)N), 154.3 (HNC), 154.2 (NC(NH<sub>2</sub>)N), 153.1 (*ipso* to OCH<sub>3</sub>), 148.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 140.2 (CHNC(NH<sub>2</sub>)N), 139.6 (*ipso* to NH), 135.4 (*ipso* to CH<sub>2</sub>O),

132.8 (*para* to CH<sub>2</sub>O), 132.1 (*para* to NH), 124.9 (*ipso* to C=O), 123.7 (*para* to C=O), 120.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 120.0 (*meta* to C=O and *ortho* to NH), 115.1 (*ortho* to C=O and *meta* to NH), 109.0 (CH<sub>2</sub>CC(NH<sub>2</sub>)=N), 108.0 (C(=O)CH), 106.3 (*meta* to CH<sub>2</sub>O), 72.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 56.0 (OCH<sub>3</sub>), 33.3 (HNCCH<sub>2</sub>), 32.1 (CCH<sub>2</sub>C), 31.2 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 29.1 (CH<sub>2</sub>CH<sub>2</sub>O), 28.3 - 28.6 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 24.7 (CH=CCH<sub>2</sub>), 22.1 (CH<sub>3</sub>CH<sub>2</sub>), 14.0 (CH<sub>3</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 641.3557, [M+H]<sup>+</sup> found, [C<sub>35</sub>H<sub>45</sub>N<sub>8</sub>O<sub>4</sub>]<sup>+</sup> 641.3558

The compound has not been reported previously.

### 9.33 2-(6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(1*H*)-one **89**



50% water/*t*-BuOH (1 mL) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71** (14.2 mg, 39.8 μmol, 1 eq.) and 2-(6-azidoethyl)-3-hydroxyquinolin-4(1*H*)-one **30** (11.4 mg, 39.8 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (1.25 mg, 5 μmol, 0.125 eq. 50 mM), THPTA (2.18 mg, 5 μmol, 0.125 eq. 50 mM) and sodium ascorbate (1.98 mg, 10 μmol, 0.25 eq., 100 mM) in water (100 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then MeOH (1 mL) was added and the reaction mixture was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **89** was obtained as a pale brown amorphous solid (4.7 mg, 7.3 μmol, 18%).

**TLC**  $R_f$  = 0.21 (20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2925 (C-H), 2853 (C-H), 1660 (pyrimidine), 1639 (pyrimidine and PQS C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 11.53 (br s, 1 H, NH), 8.09 (d,  $J$  = 8.0 Hz, 1 H, *ortho* to C=O), 7.83 (s, 1 H, NCH=CCH<sub>2</sub>), 7.48 - 7.57 (m, 3 H, *para* to C=O, *ortho* to NH and CHN=CNH<sub>2</sub>), 7.21 (ddd,  $J$  = 8.0, 6.3, 1.5 Hz, 1 H, *para* to NH), 6.55 (s, 2 H, *meta* to CH<sub>2</sub>), 4.28 (t,  $J$  = 7.1 Hz, 2 H, CH<sub>2</sub>N), 3.80 (t,  $J$  = 6.2 Hz, 2 H, CH<sub>2</sub>O), 3.70 (s, 6 H, CH<sub>3</sub>), 3.53 (d,  $J$  = 0.3 Hz, 2 H, CCH<sub>2</sub>C), 2.73 (t,  $J$  = 7.5 Hz, 2 H, HNCCH<sub>2</sub>), 2.64 (t,  $J$  = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 1.80 (quin,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N), 1.73 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.66 (quin,  $J$  = 7.2 Hz, 2 H, HNCCH<sub>2</sub>CH<sub>2</sub>), 1.62 (quin,  $J$  = 6.8 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>O), 1.33 - 1.40 (m, 2 H, HNCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.27 - 1.32 (m, 2 H, HNCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)

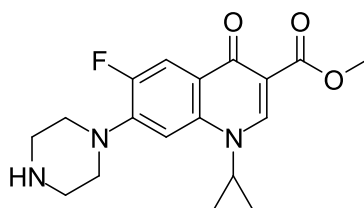
**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 168.9 (C=O), 162.5 (CC(NH<sub>2</sub>)N), 162.5 (NC(NH<sub>2</sub>)N), 152.9 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.8 (CH=CCCH<sub>2</sub>CH<sub>2</sub>), 137.7 (COH), 137.3 (*para* to OH), 135.4 (HNC), 135.1 (*para* to CH<sub>2</sub>O), 134.8 (*ipso* to CH<sub>2</sub>O), 129.9 (*para* to C=O), 124.4 (*ortho* to C=O and *meta* to NH), 122.1 (*ipso* to C=O), 121.5 (*para* to NH), 121.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 117.7 (*meta* to C=O and *ortho* to

NH), 106.2 ( $\text{CH}_2\text{C}(\text{NH}_2)=\text{N}$ ), 105.8 (*meta* to  $\text{CH}_2\text{O}$ ), 71.9 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 55.8 ( $\text{OCH}_3$ ), 49.0 ( $\text{CH}_2\text{N}$ ), 32.8 ( $\text{CCH}_2\text{C}$ ), 29.5 ( $\text{CH}_2\text{CH}_2\text{N}$ ), 29.0 ( $\text{CH}_2\text{CH}_2\text{O}$ ), 28.1 ( $\text{HNCCCH}_2\text{CH}_2\text{CH}_2$ ), 27.9 ( $\text{HNCCH}_2$ ), 27.6 ( $\text{HNCCH}_2\text{CH}_2$ ), 25.6 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 25.4 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 24.6 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 643.3365,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{34}\text{H}_{43}\text{N}_8\text{O}_5]^+$  requires 643.3351

The compound has not been reported previously.

### 9.34 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **151**



Ciprofloxacin **24** (10.0 g, 30 mmol, 1 eq.) and *para*-toluenesulfonic acid (8.60 mg, 44.5 mmol, 1.5 eq.) were refluxed in methanol (500 mL) for 72 h. The mixture was cooled to room temperature and  $\text{NaHCO}_3$  (sat., aq., 100 mL) and water (300 mL) were added. The product was extracted with  $\text{CH}_2\text{Cl}_2$  (2×400 mL). The combined organic fractions were dried over  $\text{MgSO}_4$  and evaporated under reduced pressure. **151** was obtained as a white amorphous solid (9.16 g, 26.5 mmol, 83%).

**TLC**  $R_f$  = 0.13 (5% MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2948 (C-H), 2835 (C-H), 1721 (ester C=O), 1617 (quinolone C=O)

**$^1\text{H}$  NMR** (400 MHz, MeOD)  $\delta$  / ppm = 8.55 (s, 1 H, *ortho* to  $\text{C}(=\text{O})\text{OCH}_3$ ), 7.71 (d,  $J$  = 13.5 Hz, 1 H, *ortho* to F), 7.41 (d,  $J$  = 7.2 Hz, 1 H, *meta* to F), 3.83 (s, 3 H,  $\text{CH}_3$ ), 3.62 (tt,  $J$  = 7.4, 3.5 Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.24 - 3.29 (m, 4 H,  $\text{HN}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 3.02 - 3.10 (m, 4 H,  $\text{HN}(\text{CH}_2)\text{CH}_2$ ), 1.31 - 1.38 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.12 - 1.20 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

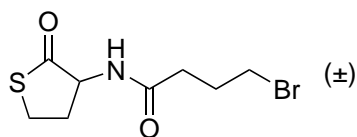
**$^{13}\text{C}$  NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.2 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OCH}_3$ ), 166.8 ( $\text{C}(=\text{O})\text{OCH}_3$ ), 154.9 (d,  $J$  = 248.0 Hz, *ipso* to F), 150.1 ( $\text{C}=\text{CC}(=\text{O})\text{OCH}_3$ ), 146.6 (d,  $J$  = 10.4 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.3 (d,  $J$  = 6.9 Hz, *para* to piperazine), 113.0 (d,  $J$  = 23.4 Hz, *ortho* to C=O and *ortho* to F), 110.1 ( $\text{CC}(=\text{O})\text{OCH}_3$ ), 107.1 (d,  $J$  = 3.5 Hz, *meta* to C=O and *meta* to F), 52.3 ( $\text{CH}_3$ ), 51.7 ( $\text{HN}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 51.6 ( $\text{HN}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 46.5 ( $\text{HN}(\text{CH}_2)\text{CH}_2$ ), 36.4 ( $\text{NCH}(\text{CH}_2)_2$ ), 8.7 ( $\text{NCH}(\text{CH}_2)_2$ )

**$^{19}\text{F}$  NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.8 (s, ciprofloxacin F)

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 346.1569,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{18}\text{H}_{21}\text{FN}_3\text{O}_3]^+$  requires 346.1567

The data are consistent with the literature.<sup>216</sup>

### 9.35 4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **153**



3-Aminodihydrothiophen-2(3*H*)-one hydrochloride **152** (15.0 g, 97.6 mmol, 1 eq.) and NaHCO<sub>3</sub> (16.4 g, 195 mmol, 2 eq.) were added to CH<sub>2</sub>Cl<sub>2</sub> (150 mL) and water (150 mL). 4-Bromobutyryl chloride **56** (11.3 mL, 107 mmol, 1.1 eq.) was added dropwise over 45 min at 0 °C and the mixture was stirred for a further 1 h. The organic layer was separated and the aqueous layer was extracted with a second portion of CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The combined organic layers were dried over MgSO<sub>4</sub> and evaporated under reduced pressure. **153** was obtained as a white, amorphous solid (22.7 g, 85.8 mmol, 88%).

**TLC**  $R_f$  = 0.19 (50% EtOAc/PE)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3266 (amide N-H), 3063 (amide N-H), 1694 (thiolactone C=O), 1651 (amide C=O)

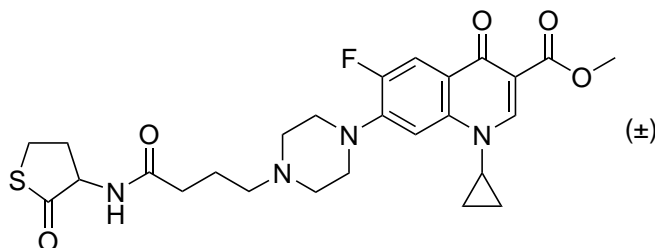
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.08 (d,  $J$  = 6.1 Hz, 1 H, NH), 4.54 (dt,  $J$  = 12.9, 6.5 Hz, 1 H, CHNH), 3.49 (t,  $J$  = 6.4 Hz, 2 H, CH<sub>2</sub>Br), 3.37 (ddd,  $J$  = 12.2, 11.5, 5.3 Hz, 1 H, SCHH), 3.26 (ddd,  $J$  = 11.5, 6.9, 1.3 Hz, 1 H, SCHH), 2.91 (dddd,  $J$  = 12.5, 6.7, 5.3, 1.3 Hz, 1 H, SCH<sub>2</sub>CHH), 2.45 (t,  $J$  = 7.4 Hz, 1 H, C(=O)CHH), 2.45 (t,  $J$  = 6.8 Hz, 1 H, C(=O)CHH), 2.20 (quin,  $J$  = 6.7 Hz, 1 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.96 (dddd,  $J$  = 12.7, 12.5, 12.2, 7.0 Hz, 1 H, SCH<sub>2</sub>CHH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 205.4 (SC(=O)), 172.1 (NHC(=O)), 59.4 (CHNH), 34.1 (C(=O)CH<sub>2</sub>), 33.1 (CH<sub>2</sub>Br), 31.8 (SCH<sub>2</sub>CH<sub>2</sub>), 28.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 27.5 (SCH<sub>2</sub>)

**LRMS** (AP+)  $m/z$  / Da = 266.1, [M+H]<sup>+</sup> found, [C<sub>8</sub>H<sub>12</sub>BrNO<sub>2</sub>S]<sup>+</sup> requires 266.0

The compound has been synthesised previously<sup>61,149</sup> but characterisation was not published.

### 9.36 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **154**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **151** (50 mg, 0.145 mmol, 1 eq.), 4-bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **153** (34.5 mg, 0.145 mmol, 1 eq.) and K<sub>2</sub>CO<sub>3</sub> (20 mg, 0.145 mmol, 1 eq.) were stirred in acetonitrile (2 mL) at 50 °C under argon. After 24 h a further portion of **153** (34.5 mg, 0.145 mmol, 1 eq.) was added. After another 24 h a further portion was added (69.0 mg, 0.290 mmol, 2 eq.). After another 24 h the temperature was raised so the mixture was at reflux. After a



final 24 h the precipitate was filtered off and the filtrate was purified by column chromatography (SiO<sub>2</sub>, 5-10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) followed by preparative HPLC (5-95% acetonitrile/water over 20 min). **154** was obtained as a pale cream amorphous solid (9.4 mg, 0.018 mmol, 12%).

**TLC**  $R_f$  = 0.47 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2944 (C-H), 2832 (C-H), 1722 (ester C=O), 1700 (thiolactone C=O), 1670 (amide C=O), 1617 (quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, MeOD)  $\delta$  / ppm = 8.53 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.68 (d,  $J$  = 13.4 Hz, 1 H, *ortho* to F), 7.41 (d,  $J$  = 7.3 Hz, 1 H, *meta* to F), 4.67 (dd,  $J$  = 12.9, 6.9 Hz, 1 H, CHNH), 3.83 (s, 3 H, OCH<sub>3</sub>), 3.61 (tt,  $J$  = 6.9, 4.1 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.39 - 3.49 (m, 1 H, SCHH), 3.26 - 3.33 (m, 5 H, SCHH and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.93 - 3.03 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.79 (br. t,  $J$  = 7.2, 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.59 (dddd,  $J$  = 12.4, 6.9, 5.4, 1.4 Hz, 1 H, SCH<sub>2</sub>CHH), 2.39 (t,  $J$  = 7.20 Hz, 1 H, C(=O)CHH), 2.38 (t,  $J$  = 6.94 Hz, 1 H, C(=O)CHH), 2.18 (qd,  $J$  = 12.4, 7.0 Hz, 1 H, SCH<sub>2</sub>CHH), 1.97 (quin,  $J$  = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.32 - 1.37 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.13 - 1.19 (m, 2 H, NCH(CHH)<sub>2</sub>)

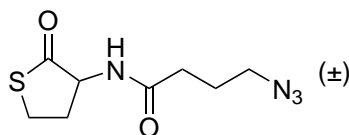
**<sup>13</sup>C NMR** (126 MHz, MeOD)  $\delta$  / ppm = 207.0 (SC(=O)), 175.7 (NHC(=O)), 175.1 (C(=O)CC(=O)OCH<sub>3</sub>), 166.6 (C(=O)OCH<sub>3</sub>), 154.7 (d,  $J$  = 249.0 Hz, *ipso* to F), 150.2 (s, CH=CC(=O)OCH<sub>3</sub>), 145.6 (d,  $J$  = 10.6 Hz, *ipso* to piperazine), 139.8 (*para* to F), 123.5 (d,  $J$  = 6.9 Hz, *para* to piperazine), 113.1 (d,  $J$  = 23.6 Hz, *ortho* to C=O and *ortho* to F), 110.0 (CC(=O)OCH<sub>3</sub>), 107.4 (*meta* to C=O and *meta* to F), 60.2 (CHNH), 58.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 53.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.3 (OCH<sub>3</sub>), 50.1 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 50.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.5 (NCH(CH<sub>2</sub>)<sub>2</sub>), 34.5 (C(=O)CH<sub>2</sub>), 31.7 (SCH<sub>2</sub>CH<sub>2</sub>), 28.1 (SCH<sub>2</sub>), 22.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 8.7 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -125.4 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 531.2083, [M+H]<sup>+</sup> found, [C<sub>26</sub>H<sub>32</sub>FN<sub>4</sub>O<sub>5</sub>S]<sup>+</sup> requires 531.2077

The compound has been synthesised previously.<sup>61,149</sup> Only HRMS characterisation was published, and this agrees with the result above.

### 9.37 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **155**



4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **153** (6.00 g, 27.0 mmol, 1 eq.) and NaN<sub>3</sub> (3.51 g, 54.1 mmol, 2 eq.) were refluxed in acetonitrile (120 mL) for 1.5 h. The solvent was evaporated under reduced pressure and the residue was partitioned between water (150 mL) and CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2×150 mL) and the combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **155** was obtained as a yellow, sticky solid (4.60 g, 20.1 mmol, 89%).

**TLC**  $R_f$  = 0.19 (50% EtOAc/PE)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3286 (N-H), 2964 (C-H), 2100 (azide), 1697 (thiolactone C=O), 1647 (amide C=O)

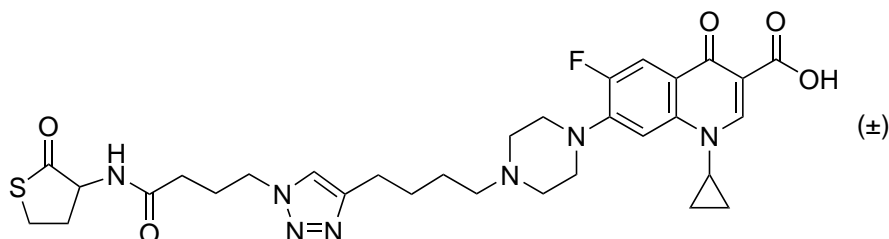
**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 6.71 (d,  $J$  = 7.3 Hz, 1 H, NH), 4.54 (dt,  $J$  = 13.0, 7.0 Hz, 1 H, CHNH), 3.30 (t,  $J$  = 6.7 Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 3.31 (td,  $J$  = 11.7, 5.3 Hz, 1 H, SCHH), 3.19 (ddd,  $J$  = 11.3, 7.0, 1.2 Hz, 1 H, SCHH), 2.70 (dddd,  $J$  = 12.4, 6.8, 5.3, 1.2 Hz, 1 H,  $\text{SCH}_2\text{CHH}$ ), 2.29 (t,  $J$  = 7.5 Hz, 1 H,  $\text{C(=O)CHH}$ ), 2.28 (t,  $J$  = 7.1 Hz, 1 H,  $\text{C(=O)CHH}$ ), 1.97 (qd,  $J$  = 12.4, 7.0 Hz, 1 H,  $\text{SCH}_2\text{CHH}$ ), 1.85 (quin,  $J$  = 6.9 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 205.4 ( $\text{SC(=O)}$ ), 172.3 ( $\text{NHC(=O)}$ ), 59.4 ( $\text{CHNH}$ ), 50.6 ( $\text{CH}_2\text{N}_3$ ), 32.8 ( $\text{C(=O)CH}_2$ ), 31.8 ( $\text{SCH}_2\text{CH}_2$ ), 27.5 ( $\text{SCH}_2$ ), 24.6 ( $\text{C(=O)CH}_2\text{CH}_2$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 251.0565,  $[\text{M}+\text{Na}]^+$  found,  $[\text{C}_8\text{H}_{12}\text{N}_4\text{NaO}_2\text{S}]^+$  requires 251.0573

The compound has not been reported previously.

**9.38 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **156****



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (15 mg, 36.7  $\mu\text{mol}$ , 1 eq.) and 4-azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **155** (12.5 mg, 55.1  $\mu\text{mol}$ , 1.5 eq.) were dissolved in 1:9:10 water/*t*-BuOH/DMSO (3 mL), and the mixture was degassed by bubbling  $\text{N}_2$  through it. A solution of  $\text{CuSO}_4$  and THPTA (182  $\mu\text{L}$ , 18.2  $\mu\text{mol}$ , 0.5 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (367  $\mu\text{L}$ , 36.7  $\mu\text{mol}$ , 1 eq., 100 mM, aq.). The mixture was stirred at r.t. under argon for 4 d. Water (10 mL) and 10% *i*-PrOH/ $\text{CHCl}_3$  (10 mL) were added, the organic layer was separated and the aqueous layer was extracted again with 10% *i*-PrOH/ $\text{CHCl}_3$  (2 $\times$ 10 mL). The combined organic layers were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between  $\text{NaHCO}_3$  (aq., sat., 50 mL) and 10% *i*-PrOH/ $\text{CHCl}_3$  (50 mL). The organic layer was dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **156** was obtained as a white amorphous solid (16.5 mg, 25.9  $\mu\text{mol}$ , 71%).

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 2919 (C-H), 1713 (carboxylic acid C=O and thiolactone C=O), 1658 (amide C=O), 1627 (quinolone C=O), 1616 (triazole)

**$^1\text{H}$  NMR** (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 15.23 (br s, 1 H,  $\text{C(=O)OH}$ ), 8.66 (s, 1 H, *ortho* to  $\text{C(=O)OH}$ ), 8.23 (d,  $J$  = 8.5 Hz, 1 H, NH), 7.90 (d,  $J$  = 13.4 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H,  $\text{CH}=\text{CCH}_2$ ), 7.56 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.59 (ddd,  $J$  = 12.7, 8.4, 6.8 Hz, 1 H, CHNH), 4.31 (t,  $J$  = 7.0 Hz, 2 H,  $\text{CH}_2\text{NCH}=\text{C}$ ), 3.80 - 3.86 (6.9, 4.0 Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.34 - 3.37 (m, 1 H, SCHH), 3.32 (br t,  $J$  = 4.1 Hz,

4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.27 (ddd, *J* = 11.1, 6.9, 1.4 Hz, 1 H, SCHH), 2.64 (t, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br t, *J* = 4.7 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 - 2.44 (m, 3 H, SCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.12 (t, *J* = 7.9 Hz, 1 H, C(=O)CHH), 2.12 (t, *J* = 7.0 Hz, 1 H, C(=O)CHH), 2.04 (m, 3 H, SCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.64 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.51 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.28 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.15 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

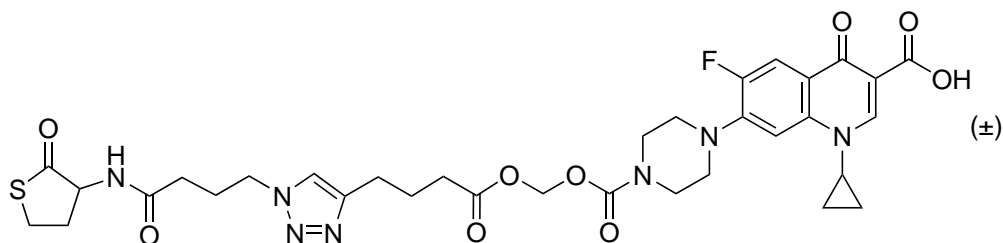
<sup>13</sup>C NMR (126 MHz, DMSO d<sub>6</sub>) δ / ppm = 205.6 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.4 (NHC(=O)), 166.0 (C(=O)OH), 153.1 (d, *J* = 249.3 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.9 (CH=CCH<sub>2</sub>), 145.3 (d, *J* = 10.1 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 (CH=CCH<sub>2</sub>), 118.6 (d, *J* = 7.7 Hz, *para* to piperazine), 111.0 (d, *J* = 23.3 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.4 (d, *J* = 2.9 Hz, *meta* to C=O and *meta* to F), 58.2 (SC(=O)CHNH), 57.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.6 (CH<sub>2</sub>NCH=C), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 31.9 (NHC(=O)CH<sub>2</sub>), 30.1 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.8 (SCH<sub>2</sub>), 25.9 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.0 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

<sup>19</sup>F NMR (376.45 MHz, MeOD) δ / ppm = -124.9 (s, ciprofloxacin F)

HRMS (ESI<sup>+</sup>) *m/z* / Da = 640.2739, [M+H]<sup>+</sup> found, [C<sub>31</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>5</sub>S]<sup>+</sup> requires 640. 2712

The compound has not been reported previously.

### 9.39 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(((4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **157**



1-Cyclopropyl-6-fluoro-7-(4-(((hex-5-ynoyloxy)methoxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **221** (203 mg, 0.407 mmol, 1 eq.), 4-azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **155** (92.8 mg, 0.407 mmol, 1 eq.), CuI (40 mg, 0.190 mmol, 0.5 eq.) and DIPEA (0.356 mL, 0.264 mg, 2.04 mmol, 5 eq.) were stirred in CH<sub>2</sub>Cl<sub>2</sub> (18.6 mL) at r.t. under Ar for 3 h. The mixture was filtered and the filtrate was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 5-10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). **157** was obtained as pale brown/yellow amorphous solid (14.7 mg, 20.2 μmol, 5%).

TLC *R<sub>f</sub>* = 0.40 (5% CH<sub>2</sub>Cl<sub>2</sub>/MeOH)

IR (neat) ν<sub>max</sub> / cm<sup>-1</sup> = 3055 (C-H), 1716 (carboxylic acid C=O and ester C=O), 1696 (carbamate C=O and thiolactone C=O), 1651 (amide C=O), 1629 (quinolone C=O)

<sup>1</sup>H NMR (400 MHz, DMSO d<sub>6</sub>) δ / ppm = 15.16 (br s, 1 H, C(=O)OH), 8.65 (s, 1 H, *ortho* to C(=O)OH), 8.21 (d, *J* = 8.5 Hz, 1 H, NH), 7.89 (d, *J* = 13.1 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH<sub>2</sub>), 7.57 (d, *J* =

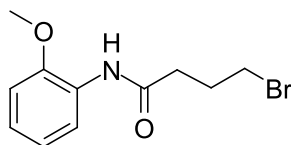
7.4 Hz, 1 H, *meta* to F), 5.74 (s, 1 H, OCH<sub>2</sub>O), 4.58 (ddd,  $J = 12.6, 8.1, 7.2$  Hz, 1 H, CHNH), 4.30 (t,  $J = 6.9$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.80 (tt,  $J = 6.9, 3.6$  Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.62 (br t,  $J = 5.2$  Hz, 4 H, C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.38 (td,  $J = 11.4, 5.5$  Hz, 1 H, SCHH), 3.34 (br. s, 4 H, C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.27 (ddd,  $J = 11.0, 6.9, 1.6$  Hz, 1 H, SCHH), 2.64 (t,  $J = 7.6$  Hz, 2 H, CH=CCH<sub>2</sub>), 2.44 (t,  $J = 7.5$  Hz, 2 H, CH<sub>2</sub>C(=O)O), 2.40 (dddd,  $J = 12.3, 6.8, 5.4, 1.4$  Hz, 1 H, SCH<sub>2</sub>CHH), 2.12 (t,  $J = 7.8$  Hz, 1 H, NHC(=O)CHH), 2.12 (t,  $J = 6.8$  Hz, 1 H, NHC(=O)CHH), 1.98 - 2.07 (m, 3 H, SCH<sub>2</sub>CHH and NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.86 (quin,  $J = 7.5$  Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.36 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.14 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

<sup>13</sup>C NMR (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 205.5 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.8 (C(=O)OCH<sub>2</sub>O), 171.3 (NHC(=O)), 165.9 (C(=O)OH), 152.8 (d,  $J = 249.7$  Hz, *ipso* to F), 152.9 (OC(=O)N), 148.1 (CH=CC(=O)OH), 146.0 (CH=CCH<sub>2</sub>), 144.9 (d,  $J = 9.6$  Hz, *ipso* to piperazine), 139.1 (*para* to F), 122.0 (CH=CCH<sub>2</sub>), 118.9 (d,  $J = 7.5$  Hz, *para* to piperazine), 111.0 (d,  $J = 23.5$  Hz, *ortho* to C=O and *ortho* to F), 106.8 (CC(=O)OH, and *meta* to C=O and *meta* to F), 80.3 (OCH<sub>2</sub>O), 58.2 (CHNH), 49.1 (C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.1 (C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 43.4 (N(CH<sub>2</sub>)CH<sub>2</sub>), 43.0 (N(CH<sub>2</sub>)CH<sub>2</sub>), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 31.8 (NHC(=O)CH<sub>2</sub>), 30.1 (SCH<sub>2</sub>CH<sub>2</sub>), 26.8 (SCH<sub>2</sub>), 25.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.2 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 24.0 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

HRMS (ESI<sup>+</sup>)  $m/z$  / Da = 728.2502, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>9</sub>S]<sup>+</sup> requires 728.2503

The compound has not been reported previously.

#### 9.40 4-Bromo-*N*-(2-methoxyphenyl)butanamide **159**



2-Methoxyaniline **158** (9.12 mL, 10.0 g, 81.2 mmol, 1 eq.) and NaHCO<sub>3</sub> (8.19 g, 97.4 mmol, 1.2 eq.) were dissolved in water (100 mL) and CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The mixture was cooled to 0 °C and 4-bromobutyryl chloride **56** (9.40 mL, 15.1 g, 81.2 mmol, 1 eq.) was added dropwise over 15 min. The mixture was stirred at 0 °C for 1.5 h, then the aqueous layer was removed. The organic layer was dried with MgSO<sub>4</sub> and purified by column chromatography (SiO<sub>2</sub>, 5-25% EtOAc/P.E.). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **159** was obtained as an initially colourless liquid which slowly turned blue then black if left out on the bench (11.0 g, 40.6 mmol, 50%).

TLC  $R_f$  = 0.16 (10% EtOAc/P.E.)

IR (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3410 (N-H), 3313 (N-H), 2962 (C-H), 2940 (C-H), 2903 (C-H), 1676 (amide C=O)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.32 (dd,  $J = 8.0, 1.7$  Hz, 1 H, *ortho* to NH), 7.85 (br s, 1 H, NH), 7.02 (td,  $J = 7.9, 1.7$  Hz, 1 H, *para* to NH), 6.93 (td,  $J = 7.7, 1.4$  Hz, 1 H, *para* to OCH<sub>3</sub>), 6.85 (dd,  $J = 8.1, 1.5$  Hz, 1 H, *ortho* to OCH<sub>3</sub>), 3.85 (s, 3 H, CH<sub>3</sub>), 3.50 (t,  $J = 6.4$  Hz, 2 H, CH<sub>2</sub>Br), 2.56 (t,  $J = 7.1$  Hz, 2 H, C(=O)CH<sub>2</sub>), 2.25 (quin,  $J = 6.7$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

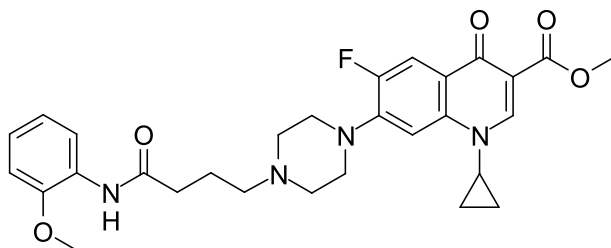
<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 169.4 (C(=O)), 147.6 (*ipso* to OCH<sub>3</sub>), 127.2 (*ipso* to NH), 123.5 (*para* to NH), 120.7 (*para* to OCH<sub>3</sub>), 119.6 (*ortho* to NH and *meta* to OCH<sub>3</sub>), 109.8 (*ortho* to OCH<sub>3</sub> and *meta*

to NH), 55.5 ( $\underline{\text{CH}_3}$ ), 35.4 ( $\text{C(=O)}\underline{\text{CH}_2}$ ), 33.1 ( $\underline{\text{CH}_2}\text{Br}$ ), 27.9 ( $\text{C(=O)}\text{CH}_2\underline{\text{CH}_2}$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 272.0287,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{11}\text{H}_{15}\text{BrNO}_2]^+$  requires 272.0286

The compound has not been reported previously.

#### 9.41 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **160**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **151** (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(2-methoxyphenyl)butanamide **159** (788 mg, 2.90 mmol, 2 eq.), DIPEA (1.28 mL, 950 mg, 7.35 mmol, 5 eq.), NaI (275 mg, 1.83 mmol, 1.3 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at 100 °C for 4 h. The mixture was dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography ( $\text{SiO}_2$ , 4% MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **160** was obtained as a bright pink amorphous solid (79.7 mg, 0.149 mmol, 10%).

**TLC**  $R_f$  = 0.40 (10% MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2947 (C-H), 2834 (C-H), 1719 (ester C=O), 1685 (amide C=O), 1617 (quinolone C=O)

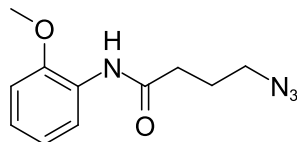
**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$   $d_1$ )  $\delta$  / ppm = 8.48 (s, 1 H, *ortho* to  $\text{C(=O)OCH}_3$ ), 8.36 (d,  $J$  = 7.9 Hz, 1 H, *ortho* to NH), 7.87 - 7.99 (m, 2 H, *ortho* to F and NH), 7.19 (d,  $J$  = 6.5 Hz, 1 H, *meta* to F), 7.01 (t,  $J$  = 7.5 Hz, 1 H, *para* to NH), 6.93 (t,  $J$  = 7.7 Hz, 1 H, *para* to  $\text{OCH}_3$ ), 6.85 (d,  $J$  = 7.9 Hz, 1 H, *ortho* to  $\text{OCH}_3$ ), 3.88 (s, 3 H,  $\text{C(=O)OCH}_3$ ), 3.85 (s, 3 H, aromatic  $\text{OCH}_3$ ), 3.41 (tt,  $J$  = 6.9, 4.0 Hz, 1 H,  $\text{NCH}(\underline{\text{CH}_2})_2$ ), 3.25 (br t,  $J$  = 5.0, 5.0 Hz, 4 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2}\underline{\text{CH}_2})\text{CH}_2\text{CH}_2$ ), 2.67 (br t,  $J$  = 5.0 Hz, 4 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2}\underline{\text{CH}_2})$ ), 2.53 (t,  $J$  = 7.0 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.47 (t,  $J$  = 7.1 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.97 (quin,  $J$  = 6.8 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.25 - 1.33 (m, 2 H,  $\text{NCH}(\underline{\text{CHH}})_2$ ), 1.07 - 1.14 (m, 2 H,  $\text{NCH}(\underline{\text{CHH}})_2$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$   $d_1$ )  $\delta$  / ppm = 172.9 ( $\underline{\text{C}}(\text{=O})\text{CC(=O)OCH}_3$ ), 170.8 ( $\text{NHC(=O)}$ ), 166.2 ( $\underline{\text{C}}(\text{=O})\text{OCH}_3$ ), 153.3 (d,  $J$  = 248.0 Hz, *ipso* to F), 148.2 ( $\underline{\text{C}}=\text{CC(=O)OCH}_3$ ), 147.6 (*ipso* to  $\text{OCH}_3$ ), 144.4 (d,  $J$  = 10.4 Hz, *ipso* to piperazine), 137.9 (*para* to F), 127.6 (*ipso* to NH), 123.4 (*para* to NH), 122.7 (d,  $J$  = 7.8 Hz, *para* to piperazine), 121.0 (*para* to  $\text{OCH}_3$ ), 119.7 (*ortho* to NH and *meta* to  $\text{OCH}_3$ ), 113.0 (d,  $J$  = 22.5 Hz, *ortho* to C=O and *ortho* to F), 109.8 (*ortho* to  $\text{OCH}_3$  and *meta* to NH, and  $\underline{\text{CC}}(\text{=O})\text{OCH}_3$ ), 104.7 (*meta* to C=O and *meta* to F), 57.2 ( $\text{CH}_2\text{CH}_2\underline{\text{CH}_2}\text{N}$ ), 55.6 (aromatic  $\text{OCH}_3$ ), 52.7 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2}\underline{\text{CH}_2})$ ), 51.9 ( $\text{C(=O)OCH}_3$ ), 49.8 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2}\underline{\text{CH}_2})\text{CH}_2\text{CH}_2$ ), 49.8 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\underline{\text{CH}_2}$ ), 35.5 ( $\underline{\text{CH}_2}\text{CH}_2\text{CH}_2\text{N}$ ), 34.5 ( $\text{NCH}(\text{CH}_2)_2$ ), 22.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 8.0 ( $\text{NCH}(\underline{\text{CH}_2})_2$ )

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 537.2523, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>34</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 537.2513

The compound has not been reported previously.

#### 9.42 4-Azido-*N*-(2-methoxyphenyl)butanamide **161**



4-Bromo-*N*-(2-methoxyphenyl)butanamide **159** (2.05 g, 7.51 mmol, 1 eq.) and NaN<sub>3</sub> (1.17 g, 18.0 mmol, 2.4 eq.) were refluxed in acetonitrile (100 mL) for 2 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 8-14% then held at 14% EtOAc/P.E.). **161** was obtained as an initially colourless liquid which slowly turned blue then black if left out on the bench (0.469 g, 2.00 mmol, 27%).

**TLC**  $R_f$  = 0.20 (25% EtOAc/P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3420 (N-H), 3330 (N-H), 2095 (azide), 1672 (amide C=O)

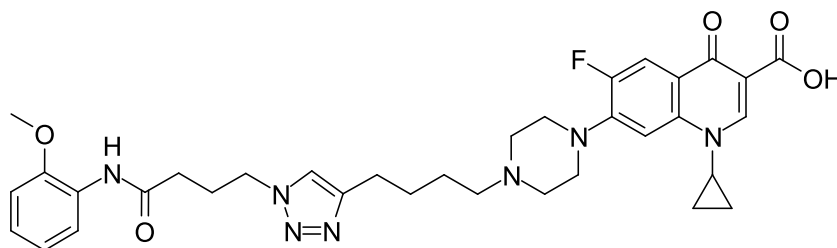
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.32 (dd,  $J$  = 7.9, 1.0 Hz, 1 H, *ortho* to NH), 7.86 (br s, 1 H, NH), 7.00 (td,  $J$  = 7.5, 1.5 Hz, 1 H, *para* to NH), 6.90 (td,  $J$  = 7.7, 1.1 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.83 (dd,  $J$  = 8.1, 1.4 Hz, 1 H, *ortho* to OCH<sub>3</sub>), 3.81 (s, 3 H, CH<sub>3</sub>), 3.33 (t,  $J$  = 6.7 Hz, 2 H, CH<sub>2</sub>Br), 2.42 (t,  $J$  = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>), 1.94 (quin,  $J$  = 6.9 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 169.5 (C(=O)), 147.6 (*ipso* to OCH<sub>3</sub>), 127.1 (*ipso* to NH), 123.4 (*para* to NH), 120.5 (*para* to OCH<sub>3</sub>), 119.5 (*ortho* to NH and *meta* to OCH<sub>3</sub>), 109.6 (*ortho* to OCH<sub>3</sub> and *meta* to NH), 55.2 (CH<sub>3</sub>), 50.3 (CH<sub>2</sub>N<sub>3</sub>), 33.9 (C(=O)CH<sub>2</sub>), 24.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 257.1010, [M+H]<sup>+</sup> found, [C<sub>11</sub>H<sub>14</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 257.1014

The data are consistent with the literature.<sup>240</sup>

#### 9.43 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **162**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (24.1 mg, 58.6  $\mu$ mol, 1 eq.) and 4-azido-*N*-(2-methoxyphenyl)butanamide **161** (13.7 mg, 58.5  $\mu$ mol, 1 eq.) were dissolved in water (3 mL), *t*-BuOH (9 mL) and CH<sub>2</sub>Cl<sub>2</sub> (9 mL), and the mixture was degassed by bubbling through N<sub>2</sub>. A solution of CuSO<sub>4</sub> and THPTA (117  $\mu$ l, 5.85  $\mu$ mol, 0.1 eq., 50 mM, aq.) was added, followed by a solution of sodium ascorbate (234  $\mu$ l, 11.7  $\mu$ mol, 0.2 eq., 50 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (25 mL), CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and MeOH (5 mL) were added and the organic layer was separated off, dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 3-23% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **162** was obtained as a clear amorphous solid (14.7 mg, 22.8  $\mu$ mol, 39%).

**TLC**  $R_f$  = 0.28 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2927 (C-H), 2847 (C-H), 1723 (carboxylic acid C=O), 1682 (amide C=O), 1626 (quinolone C=O), 1613 (triazole)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 15.05 (br s, 1 H, C(=O)OH), 8.76 (s, 1 H, *ortho* to C(=O)OH), 8.31 (dd,  $J$  = 8.0, 1.7 Hz, 1 H, *ortho* to NH), 8.00 (d,  $J$  = 13.0 Hz, 1 H, *ortho* to F), 7.83 (br s, 1 H, NH), 7.37 (s, 1 H, CH=CCH<sub>2</sub>), 7.35 (d,  $J$  = 7.2 Hz, 1 H, *meta* to F), 7.04 (td,  $J$  = 7.7, 1.7 Hz, 1 H, *para* to NH), 6.95 (td,  $J$  = 7.8, 1.5 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.88 (dd,  $J$  = 8.1, 1.4 Hz, 1 H, *ortho* to OCH<sub>3</sub>), 4.47 (t,  $J$  = 6.7 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.88 (s, 3 H, CH<sub>3</sub>), 3.54 (tt,  $J$  = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.35 (br t,  $J$  = 4.7 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.76 (t,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.66 (t,  $J$  = 4.7 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.47 (t,  $J$  = 7.3 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.44 (t,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.32 (quin,  $J$  = 6.7 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.75 (quin,  $J$  = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.61 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.35 - 1.42 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.17 - 1.22 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

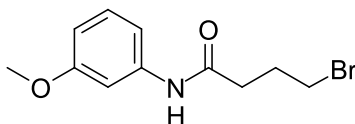
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 177.1 (C(=O)CC(=O)OH), 169.5 (NHC(=O)), 167.0 (C(=O)OH), 153.7 (d,  $J$  = 251.4 Hz, *ipso* to F), 148.1 (CH=CCH<sub>2</sub>), 147.8 (*ipso* to OCH<sub>3</sub>), 147.3 (C=CC(=O)OH), 145.9 (d,  $J$  = 10.4 Hz, *ipso* to piperazine), 139.1 (*para* to F), 127.3 (*ipso* to NH), 123.9 (*para* to NH), 121.0 (*para* to OCH<sub>3</sub>), 120.9 (CH=CCH<sub>2</sub>), 119.7 (*para* to piperazine, and *ortho* to NH and *meta* to OCH<sub>3</sub>), 112.4 (d,  $J$  = 23.4 Hz, *ortho* to C=O and *ortho* to F), 109.9 (*ortho* to OCH<sub>3</sub> and *meta* to NH), 108.1 (CC(=O)OH), 104.7 (*meta* to C=O and *meta* to F), 58.1 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 55.6 (CH<sub>3</sub>), 52.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 35.2 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 27.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 8.2 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = -120.7 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 646.3132, [M+H]<sup>+</sup> found, [C<sub>34</sub>H<sub>41</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 646.3153

The compound has not been reported previously.

#### 9.44 4-Bromo-*N*-(3-methoxyphenyl)butanamide **164**



3-Methoxyaniline **163** (3.04 mL, 3.33 g, 27.1 mmol, 1 eq.) and NaHCO<sub>3</sub> (2.73 g, 32.5 mmol, 1.2 eq.) were dissolved in water (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (30 mL). The mixture was cooled to 0 °C and 4-bromobutyryl chloride **56** (3.13 mL, 5.03 g, 27.1 mmol, 1 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h, then the aqueous layer was removed. The organic layer was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-100% EtOAc/P.E.). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **164** was obtained as a pale pink amorphous solid (3.66 g, 13.5 mmol, 50%).

**TLC**  $R_f$  = 0.18 (25% EtOAc/P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 1671 (amide C=O)

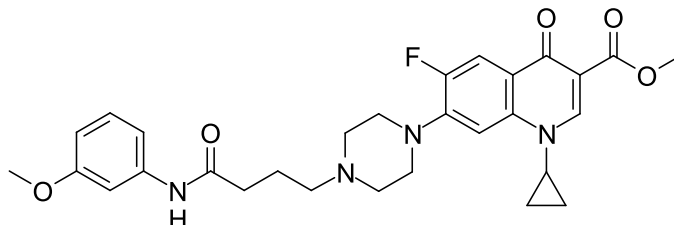
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.45 (s, 1 H, NH), 7.27 (t,  $J$  = 2.2 Hz, 1 H, *ortho* to OCH<sub>3</sub> and *ortho* to NH), 7.14 (t,  $J$  = 8.1 Hz, 1 H, *meta* to OCH<sub>3</sub> and *meta* to NH), 7.02 (d,  $J$  = 8.3 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.62 (dd,  $J$  = 8.2, 2.1 Hz, 1 H, *para* to NH), 3.71 (s, 3 H, CH<sub>3</sub>), 3.42 (t,  $J$  = 6.5 Hz, 2 H, CH<sub>2</sub>Br), 2.51 (t,  $J$  = 6.9 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.19 (quin,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 170.3 (C(=O)), 159.9 (*ipso* to OCH<sub>3</sub>), 139.0 (*ipso* to NH), 129.5 (*meta* to OCH<sub>3</sub> and *meta* to NH), 112.1 (*para* to OCH<sub>3</sub>), 109.9 (*para* to NH), 105.7 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 55.2 (CH<sub>3</sub>), 35.3 (C(=O)CH<sub>2</sub>), 33.2 (CH<sub>2</sub>Br), 28.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) The compound does not ionise.

The compound has not been reported previously.

#### 9.45 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((3-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **165**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **151** (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(3-methoxyphenyl)butanamide **164** (788 mg, 2.90 mmol, 2 eq.), DIPEA (1.28 mL, 950 mg, 7.35 mmol, 5 eq.), NaI (275 mg, 1.83 mmol, 1.3 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at 100 °C for 4 h. The mixture was evaporated under reduced pressure and partitioned between CH<sub>2</sub>Cl<sub>2</sub>



(50 mL) and water (50 mL). The organic layer was separated off and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The combined organic layers were dried with MgSO<sub>4</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-4% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **165** was obtained as an off-white amorphous solid (81.7 mg, 0.152 mmol, 11%).

**TLC**  $R_f$  = 0.38 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3271 (amide N-H) 2944 (C-H), 2817 (C-H), 1730 (ester C=O), 1682 (amide C=O), 1614 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 8.56 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 8.06 (d,  $J$  = 13.3 Hz, 1 H, *ortho* to F), 8.02 (br s, 1 H, NH), 7.34 (t,  $J$  = 1.7 Hz, 1 H, *ortho* to OCH<sub>3</sub> and *ortho* to NH), 7.25 (d,  $J$  = 7.0 Hz, 1 H, *meta* to F), 7.20 (t,  $J$  = 8.2 Hz, 1 H, *meta* to OCH<sub>3</sub> and *meta* to NH), 6.98 (dd,  $J$  = 7.8, 1.7 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.65 (dd,  $J$  = 8.2, 2.1 Hz, 1 H, *para* to NH), 3.93 (s, 3 H, C(=O)OCH<sub>3</sub>), 3.80 (s, 3 H, aromatic OCH<sub>3</sub>), 3.42 (tt,  $J$  = 6.8, 3.7 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.31 (br t,  $J$  = 4.3 Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.73 (br t,  $J$  = 4.5 Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.58 (t,  $J$  = 6.5 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.48 (t,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.00 (quin,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.29 - 1.36 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.11 - 1.17 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

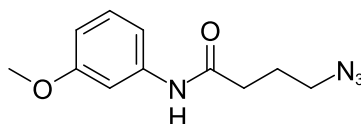
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.1 (C(=O)CC(=O)OCH<sub>3</sub>), 170.9 (NHC(=O)), 166.3 (C(=O)OCH<sub>3</sub>), 160.1 (*ipso* to OCH<sub>3</sub>), 153.3 (d,  $J$  = 250.1 Hz, *ipso* to F), 148.4 (C=CC(=O)OCH<sub>3</sub>), 144.1 (d,  $J$  = 10.1 Hz, *ipso* to piperazine), 139.4 (*ipso* to NH), 138.0 (*para* to F), 129.6 (*meta* to NH and *meta* to OCH<sub>3</sub>), 123.3 (d,  $J$  = 6.4 Hz, *para* to piperazine), 113.4 (d,  $J$  = 23.3 Hz, *ortho* to C=O and *ortho* to F), 111.8 (*para* to OCH<sub>3</sub>), 110.0 (CC(=O)OCH<sub>3</sub>), 109.8 (*para* to NH), 105.5 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 105.0 (*meta* to C=O and *meta* to F), 57.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 55.3 (aromatic OCH<sub>3</sub>), 52.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.1 (C(=O)OCH<sub>3</sub>), 49.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 35.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 34.6 (NCH(CH<sub>2</sub>)<sub>2</sub>), 21.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 8.2 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -123.5 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 537.2500, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>34</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 537.2513

The compound has not been reported previously.

#### 9.46 4-Azido-*N*-(3-methoxyphenyl)butanamide **166**



4-Bromo-*N*-(3-methoxyphenyl)butanamide **164** (2.05 g, 7.51 mmol, 1 eq.) and NaN<sub>3</sub> (1.17 g, 18.0 mmol, 2.4 eq.) were refluxed in acetonitrile (100 mL) for 7 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-100% EtOAc/P.E.). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **166** was obtained as a straw-coloured liquid (0.294 g, 1.25 mmol, 17%).

**TLC**  $R_f = 0.37$  (50% EtOAc/P.E.)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1} = 3298$  (N-H), 2095 (azide), 1662 (amide C=O)

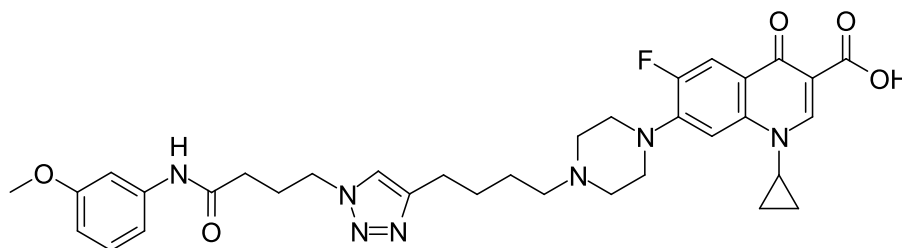
**$^1\text{H}$  NMR** (400 MHz, MeOD)  $\delta$  / ppm = 8.63 (br s, 1 H, NH), 7.26 (t,  $J = 2.3$  Hz, 1 H, *ortho* to  $\text{OCH}_3$  and *ortho* to NH), 7.15 (t,  $J = 8.1$  Hz, 1 H, *meta* to  $\text{OCH}_3$  and *meta* to NH), 7.01 (dd,  $J = 7.8, 1.6$  Hz, 1 H, *para* to  $\text{OCH}_3$ ), 6.63 (dd,  $J = 8.2, 1.9$  Hz, 1 H, *para* to NH), 3.69 (s, 3 H,  $\text{CH}_3$ ), 3.28 (t,  $J = 6.7$  Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.39 (t,  $J = 7.4$  Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2$ ), 1.91 (quin,  $J = 7.0$  Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ )

**$^{13}\text{C}$  NMR** (101 MHz, MeOD)  $\delta$  / ppm = 170.8 ( $\text{C}(=\text{O})$ ), 159.6 (*ipso* to  $\text{OCH}_3$ ), 138.9 (*ipso* to NH), 129.2 (*meta* to  $\text{OCH}_3$  and *meta* to NH), 112.3 (*para* to  $\text{OCH}_3$ ), 109.5 (*para* to NH), 106.0 (*ortho* to  $\text{OCH}_3$  and *ortho* to NH), 54.8 ( $\text{CH}_3$ ), 50.4 ( $\text{CH}_2\text{N}_3$ ), 33.6 ( $\text{C}(=\text{O})\text{CH}_2$ ), 24.4 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ )

**HRMS** ( $\text{ESI}^+$ ) The compound does not ionise.

The compound has not been reported previously.

**9.47 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **167****



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (24.1 mg, 58.6  $\mu\text{mol}$ , 1 eq.) and 4-azido-*N*-(3-methoxyphenyl)butanamide **166** (13.7 mg, 58.5  $\mu\text{mol}$ , 1 eq.) were dissolved in water (1 mL), *t*-BuOH (9 mL) and  $\text{CH}_2\text{Cl}_2$  (10 mL), and the mixture was degassed by bubbling through  $\text{N}_2$ . A solution of  $\text{CuSO}_4$  and THPTA (58.5  $\mu\text{l}$ , 5.85  $\mu\text{mol}$ , 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (117  $\mu\text{l}$ , 11.7  $\mu\text{mol}$ , 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 2 h, then the solvent was removed under reduced pressure. The residue was partitioned between water (15 mL) and  $\text{CH}_2\text{Cl}_2$  (15 mL), and the aqueous layer was extracted a further four times with  $\text{CH}_2\text{Cl}_2$  (4 $\times$ 15 mL). The combined organic layers were dried with  $\text{MgSO}_4$ , dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography ( $\text{SiO}_2$ , 0-10% MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **167** was obtained as a clear amorphous solid (1.9 mg, 2.9  $\mu\text{mol}$ , 5%).

**TLC**  $R_f = 0.22$  (10% MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1} = 2923$  (C-H), 2850 (C-H), 1726 (carboxylic acid C=O), 1685 (amide C=O), 1625 (quinolone C=O), 1612 (triazole)

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 15.23 (br s, 1 H,  $\text{C}(=\text{O})\text{OH}$ ), 9.89 (s, 1 H, NH), 8.66 (s, 1 H, *ortho* to  $\text{C}(=\text{O})\text{OH}$ ), 7.90 (d,  $J = 13.4$  Hz, 1 H, *ortho* to F), 7.88 (s, 1 H,  $\text{CH}=\text{CCH}_2$ ), 7.55 (d,  $J = 7.6$  Hz,

1 H, *meta* to F), 7.27 (t,  $J = 2.1$  Hz, 1 H, *ortho* to C=O and *ortho* to F), 7.16 (t,  $J = 8.1$  Hz, 1 H, *meta* to OCH<sub>3</sub> and *meta* to NH), 7.08 (d,  $J = 7.8$  Hz, 1 H, *para* to OCH<sub>3</sub>), 6.59 (ddd,  $J = 8.1, 2.4, 0.7$  Hz, 1 H, *para* to NH), 4.36 (t,  $J = 6.9$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.81 (tt,  $J = 6.7, 4.0$  Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.70 (s, 3 H, CH<sub>3</sub>), 3.28 - 3.32 (m, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t,  $J = 7.5$  Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (m,  $J = 4.2, 4.2$  Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.38 (t,  $J = 7.3$  Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.30 (t,  $J = 7.4$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.10 (quin,  $J = 7.1$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.64 (quin,  $J = 7.5$  Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.51 (quin,  $J = 7.2$  Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.27 - 1.33 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.15 - 1.20 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

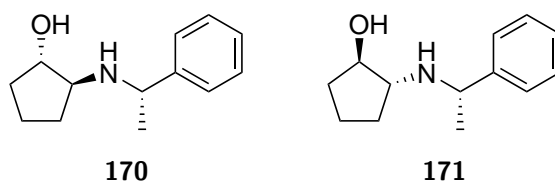
<sup>13</sup>C NMR (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.3 (C(=O)CC(=O)OH), 170.1 (NHC(=O)), 165.9 (C(=O)OH), 159.4 (*ipso* to OCH<sub>3</sub>), 153.0 (d,  $J = 248.6$  Hz, *ipso* to F), 148.0 (CH=CCH<sub>2</sub>), 146.9 (C=CC(=O)OH), 145.2 (d,  $J = 10.7$  Hz, *ipso* to piperazine), 140.3 (*para* to F), 139.2 (*ipso* to NH), 129.4 (*meta* to OCH<sub>3</sub> and *meta* to NH), 121.7 (CH=CCH<sub>2</sub>), 118.5 (d,  $J = 7.5$  Hz, *para* to piperazine), 111.3 (*para* to OCH<sub>3</sub>), 110.9 (d,  $J = 22.4$  Hz, *ortho* to C=O and *ortho* to F), 108.4 (*para* to NH), 106.7 (CC(=O)OH), 106.3 (*meta* to C=O and *meta* to F), 104.8 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 54.9 (CH<sub>3</sub>), 52.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 35.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

<sup>19</sup>F NMR (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.5 (s, ciprofloxacin F)

HRMS (ESI<sup>+</sup>)  $m/z$  / Da = 646.3159, [M+H]<sup>+</sup> found, [C<sub>34</sub>H<sub>41</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 646.3153

The compound has not been reported previously.

#### 9.48 (1*S*,2*S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol **170** and (1*R*,2*R*)-2-(((*S*)-1-phenylethyl)amino)cyclopentan-1-ol **171**



(*S*)-1-Phenylethan-1-amine **169** (7.85 mL, 7.38 g, 60.9 mmol, 1 eq.) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and stirred rapidly at 0 °C. A solution of AlMe<sub>3</sub> (31 mL, 2.0 M in heptane, 60.9 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. A solution of cyclohexene oxide **168** (5.71 mL, 5.50 g, 65.4 mmol, 1.1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was then added dropwise, and the mixture was stirred at 0 °C for a further 3 h, followed by 48 h at r.t.. The mixture was cooled to 0 °C and NaF (11 g, 262 mmol, 4.3 eq.) was added portionwise, followed by water (7.00 mL, 7.00 g, 389 mmol, 6.4 eq.) and CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The suspension was allowed to warm to r.t. and stirred for 1 h, then filtered through Celite and washed with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The filtrate was dried with K<sub>2</sub>CO<sub>3</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, 20:5:1 hexane:EtOAc:TEA). **171** was obtained as a pale yellow oil (4.08 g, 19.9 mmol, 33%). **170** was obtained as pale yellow crystals (4.48 g, 21.8 mmol, 36%).

**(1*S*,2*S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 170**

**TLC**  $R_f$  = 0.36 (15:5:1 hexane:EtOAc:TEA)

**mp**  $T$  / °C = 66-72 (hexane, EtOAc, TEA)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3150 (br, O-H), 2951 (C-H), 2868 (C-H)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 7.28 - 7.34 (m, 4 H, *ortho* and *meta* to  $\text{CHCH}_3$ ), 7.20 - 7.26 (m, 1 H, *para* to  $\text{CHCH}_3$ ), 3.86 (q,  $J$  = 6.6 Hz, 1 H,  $\text{CHCH}_3$ ), 3.85 (q,  $J$  = 6.6 Hz, 1 H,  $\text{CHOH}$ ), 2.83 (td,  $J$  = 7.6, 5.7 Hz, 1 H,  $\text{CHNH}$ ), 1.85 - 1.97 (m, 1 H,  $\text{CHHCHOH}$ ), 1.77 (dtd,  $J$  = 12.9, 7.9, 4.9 Hz, 1 H,  $\text{CHHCHNH}$ ), 1.55 - 1.68 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 1.47 - 1.55 (m, 1 H,  $\text{CHHCHOH}$ ), 1.36 (d,  $J$  = 6.6 Hz, 3 H,  $\text{CH}_3$ ), 1.12 (dq,  $J$  = 12.7, 8.1 Hz, 1 H,  $\text{CHHCHNH}$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 145.61 (*ipso* to  $\text{CHCH}_3$ ), 128.08 (*meta* to  $\text{CHCH}_3$ ), 126.61 (*para* to  $\text{CHCH}_3$ ), 126.33 (*ortho* to  $\text{CHCH}_3$ ), 77.43 ( $\text{CHOH}$ ), 64.45 ( $\text{CHNH}$ ), 56.62 ( $\text{CHCH}_3$ ), 32.01 ( $\text{CH}_2\text{CHOH}$ ), 30.56 ( $\text{CH}_2\text{CHNH}$ ), 23.30 ( $\text{CH}_3$ ), 20.06 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 206.1553,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{13}\text{H}_{20}\text{NO}]^+$  requires 206.1545

$[\alpha]_D^{20}$  /  $^{\circ}\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -23.9, lit. = -22.1 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.96, MeOH)

**(1*R*,2*R*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 171**

**TLC**  $R_f$  = 0.25 (15:5:1 hexane:EtOAc:TEA)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3300 (br, O-H), 2960 (C-H), 2870 (C-H)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 7.28 - 7.38 (m, 4 H, *ortho* and *meta* to  $\text{CHCH}_3$ ), 7.21 - 7.28 (m, 1 H, *para* to  $\text{CHCH}_3$ ), 3.83 (q,  $J$  = 6.6 Hz, 1 H,  $\text{CHCH}_3$ ), 3.78 (q,  $J$  = 7.0 Hz, 1 H,  $\text{CHOH}$ ), 2.62 (dt,  $J$  = 8.2, 7.2 Hz, 1 H,  $\text{CHNH}$ ), 1.97 (quin,  $J$  = 6.7 Hz, 1 H,  $\text{CH}_2\text{CHNH}$ ), 1.90 (quin,  $J$  = 6.9 Hz, 1 H,  $\text{CH}_2\text{CHOH}$ ), 1.56 - 1.68 (m,  $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 1.43 (dq,  $J$  = 12.5, 8.0 Hz, 1 H,  $\text{CH}_2\text{CHOH}$ ), 1.37 (d,  $J$  = 6.6 Hz, 3 H,  $\text{CH}_3$ ), 1.25 - 1.36 (m, 1 H,  $\text{CH}_2\text{CHNH}$ )

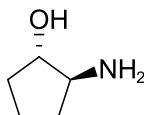
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 144.75 (*ipso* to  $\text{CHCH}_3$ ), 128.26 (*meta* to  $\text{CHCH}_3$ ), 126.72 (*para* to  $\text{CHCH}_3$ ), 126.30 (*ortho* to  $\text{CHCH}_3$ ), 77.65 ( $\text{CHOH}$ ), 63.38 ( $\text{CHNH}$ ), 56.20 ( $\text{CHCH}_3$ ), 31.74 ( $\text{CH}_2\text{CHOH}$ ), 29.22 ( $\text{CH}_2\text{CHNH}$ ), 24.58 ( $\text{CH}_3$ ), 19.57 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 206.1554,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{13}\text{H}_{20}\text{NO}]^+$  requires 206.1545

$[\alpha]_D^{20}$  /  $^{\circ}\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -92.8, lit. = -76.8 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 1.19, MeOH)

The compounds have been synthesised previously,<sup>219,220</sup> but NMR data were not published. The enantiomers of both compounds have also been synthesised previously, and the  $^1\text{H}$  NMR data for these are consistent with the the above data.<sup>221</sup>

### 9.49 (1*S*,2*S*)-2-Aminocyclopentan-1-ol **172**



(1*S*,2*S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol **170** (3.00 g, 14.6 mmol, 1 eq.), Pd(OH)<sub>2</sub> (20 wt. % on C, moistened with 50 wt. % water, 0.5 g, 0.356 mmol, 0.025 eq.) and MeOH (50 mL) were stirred in a Paar hydrogenator at r.t. and 2.5 atm for 2 days. The mixture was then filtered through Celite and evaporated under reduced pressure. **172** was obtained as a yellow oil (1.48 g, 14.6 mmol, >99%).

**TLC**  $R_f$  = 0.10 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3300 (O-H), 2969 (C-H), 2873 (C-H)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.77 (ddd,  $J$  = 6.6, 6.2, 5.6, 1 H, CHOH), 3.00 (td,  $J$  = 7.4, 5.6 Hz, 1 H, CHNH<sub>2</sub>), 2.00 (dtd,  $J$  = 13.0, 7.7, 5.6 Hz, 1 H, CHHCHNH<sub>2</sub>), 1.97 (ddt,  $J$  = 13.0, 8.7, 6.4 Hz, 1 H, CHHCHOH), 1.64 - 1.77 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.53 (ddt,  $J$  = 13.0, 9.5, 6.2 Hz, 1 H, CHHCHOH), 1.37 (ddt,  $J$  = 12.8, 8.5, 7.7 Hz, 1 H, CHHCHNH<sub>2</sub>)

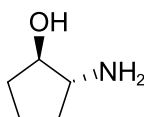
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 80.6 (CHOH), 60.7 (CHNH<sub>2</sub>), 33.2 (CH<sub>2</sub>CHOH), 32.2 (CH<sub>2</sub>CHNH<sub>2</sub>), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 102.0915, [M+H]<sup>+</sup> found, [C<sub>5</sub>H<sub>12</sub>NO]<sup>+</sup> requires 102.0913

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 33.4, lit. = 29.7 ( $c$  / g(100 mL)<sup>-1</sup> = 0.5, EtOH)

The data are consistent with the literature.<sup>220,241</sup>

### 9.50 (1*R*,2*R*)-2-Aminocyclopentan-1-ol **173**



(1*R*,2*R*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol **171** (3.90 g, 19.0 mmol, 1 eq.), Pd(OH)<sub>2</sub> (20 wt. % on C, moistened with 50 wt. % water, 1 g, 0.712 mmol, 0.04 eq.) and MeOH (50 mL) were stirred in a Paar hydrogenator at r.t. and 3 atm for 2 days. The mixture was then filtered through Celite and evaporated under reduced pressure. **173** was obtained as a yellow oil (1.92 g, 19.0 mmol, >99%).

**TLC**  $R_f$  = 0.10 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3300 (br, O-H), 2958 (C-H), 2872 (C-H)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.77 (ddd,  $J$  = 6.6, 6.2, 5.6, 1 H, CHOH), 3.00 (td,  $J$  = 7.3, 5.6

Hz, 1 H,  $\text{CHNH}_2$ ), 2.00 (dtd,  $J = 13.0, 7.7, 5.6$  Hz, 1 H,  $\text{CHHCHNH}_2$ ), 1.97 (dtd,  $J = 13.0, 8.7, 6.6$  Hz, 1 H,  $\text{CHHCHOH}$ ), 1.63 - 1.77 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 1.53 (dtd,  $J = 13.0, 9.5, 6.2$  Hz, 1 H,  $\text{CHHCHOH}$ ), 1.37 (dtd,  $J = 13.0, 8.3, 7.8$  Hz, 1 H,  $\text{CHHCHNH}_2$ )

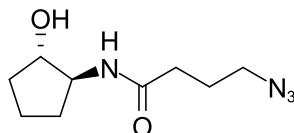
$^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta$  / ppm = 80.7 ( $\text{CHOH}$ ), 60.8 ( $\text{CHNH}_2$ ), 33.2 ( $\text{CH}_2\text{CHOH}$ ), 32.1 ( $\text{CH}_2\text{CHNH}_2$ ), 21.2 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 102.0917,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_5\text{H}_{12}\text{NO}]^+$  requires 102.0913

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = -30.9, lit. = -32.9 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 1.5, EtOH)

The data are consistent with the literature.<sup>220,241</sup>

### 9.51 4-Azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **176**



4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **193** (35.0 mg, 0.170 mmol, 1 eq.) and  $\text{NaN}_3$  (22.1 mg, 0.340 mmol, 2 eq.) were stirred in acetonitrile (2 mL) at 50  $^{\circ}\text{C}$  for 24 h. The reaction mixture was then partitioned between water (20 mL) and 10% *i*-PrOH/ $\text{CHCl}_3$  (5 mL). The aqueous layer was extracted again with 10% *i*-PrOH/ $\text{CHCl}_3$  (2 $\times$ 5 mL) and the combined organic fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **176** was obtained as white needles (16.2 mg, 0.0764 mmol, 45%).

TLC  $R_f$  = 0.35 (EtOAc)

IR (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 3287 (N-H and O-H), 2958 (C-H), 2931 (C-H), 2861 (C-H), 2095 (azide), 1642 (amide C=O)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 5.82 (br s, 1 H,  $\text{NH}$ ), 4.45 (br. s., 1 H,  $\text{OH}$ ), 3.96 (q,  $J = 6.6$  Hz, 1 H,  $\text{CHOH}$ ), 3.83 (tdd,  $J = 8.5, 6.0, 4.6$  Hz, 1 H,  $\text{CHNH}$ ), 3.37 (t,  $J = 6.4$  Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.31 (t,  $J = 7.2$  Hz, 2 H,  $\text{CH}_2\text{C=O}$ ), 2.09 - 2.19 (m, 1 H,  $\text{CHHCHNH}$ ), 1.99 - 2.06 (m, 1 H,  $\text{CHHCHOH}$ ), 1.90 - 1.97 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{N}_3$ ), 1.60 - 1.85 (m, 3 H,  $\text{CH}_2\text{CHHCHOH}$ ), 1.42 (dq,  $J = 12.8, 8.3$  Hz, 1 H,  $\text{CHHCHNH}$ )

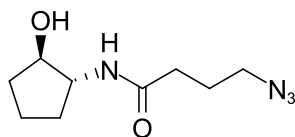
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 173.8 ( $\text{C=O}$ ), 79.7 ( $\text{CHOH}$ ), 61.0 ( $\text{CHNH}$ ), 50.7 ( $\text{CH}_2\text{N}_3$ ), 32.8 ( $\text{CH}_2\text{C=O}$ ), 32.6 ( $\text{CH}_2\text{CHOH}$ ), 30.5 ( $\text{CH}_2\text{CHNH}$ ), 24.7 ( $\text{CH}_2\text{CH}_2\text{N}_3$ ), 21.3 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 235.1178,  $[\text{M}+\text{Na}]^+$  found,  $[\text{C}_9\text{H}_{16}\text{N}_4\text{NaO}_2]^+$  requires 235.1171

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = 10.0 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.01, MeOH)

The compound has not been reported previously.

### 9.52 4-Azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **177**



4-Chloro-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **194** (200 mg, 0.972 mmol, 1 eq.) and NaN<sub>3</sub> (126 mg, 1.94 mmol, 2 eq.) were stirred in acetonitrile (4 mL) at 50 °C for 16 h. The solvent was then evaporated under reduced pressure and the residue was partitioned between water (20 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (20 mL). The aqueous layer was extracted again with 10% *i*-PrOH/CHCl<sub>3</sub> (3×20 mL) and the combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **177** was obtained as white needles (181 mg, 0.852 mmol, 88%).

**TLC**  $R_f$  = 0.35 (EtOAc)

**mp**  $T$  / °C = 56-60 (*i*-PrOH, CHCl<sub>3</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3280 (N-H and O-H), 2966 (C-H), 2875 (C-H), 2095 (azide), 1637 (amide C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.72 (d,  $J$  = 4.4 Hz, 1 H, NH), 4.82 (br. s., 1 H, OH), 3.88 (q,  $J$  = 6.6 Hz, 1 H, CHOH), 3.75 (tdd,  $J$  = 8.4, 6.6, 4.4 Hz, 1 H, CHNH), 3.28 (t,  $J$  = 6.6 Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 2.23 (t,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>C=O), 2.04 (dtd,  $J$  = 13.0, 8.0, 4.9 Hz, 1 H, CHHCHNH), 1.92 (dtd,  $J$  = 13.0, 7.6, 5.8 Hz, 1 H, CHHCHOH), 1.84 (quin,  $J$  = 7.0 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.59 - 1.77 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.54 (ddt,  $J$  = 12.7, 9.0, 6.7 Hz, 1 H, CHHCHOH), 1.39 (dq,  $J$  = 12.9, 8.4 Hz, 1 H, CHHCHNH)

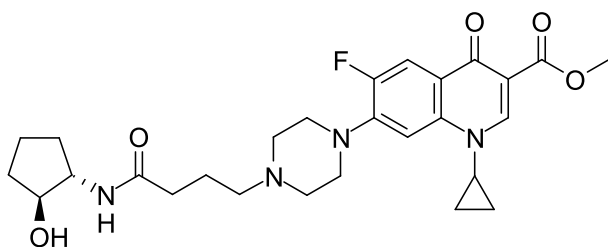
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 78.8 (CHOH), 59.9 (CHNH), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 32.5 (CH<sub>2</sub>C=O), 32.0 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 24.6 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.7 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 235.1174, [M+Na]<sup>+</sup> found, [C<sub>9</sub>H<sub>16</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 235.1171

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -10.2 ( $c$  / g(100 mL)<sup>-1</sup> = 0.5, MeOH)

The compound has not been reported previously.

### 9.53 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **178**



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **198** (52.1 mg, 95.5  $\mu$ mol, 1 eq.), (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** (19.5 mg, 193  $\mu$ mol, 2 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (29.7 mg, 155  $\mu$ mol, 1.6 eq.), 1-hydroxybenzotriazole (25.8 mg, 191  $\mu$ mol, 2 eq.) and DIPEA (33.3  $\mu$ L, 24.7 mg, 191  $\mu$ mol, 2 eq.) were dissolved in DMF (2 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of N<sub>2</sub> and the residue was purified by preparative HPLC (5-50% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The organic layer was removed and the aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2 $\times$ 5 mL). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **178** was obtained as a white amorphous solid (26.9 mg, 52.3  $\mu$ mol, 55%).

**TLC**  $R_f$  = 0.38 (30% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2938 (C-H), 1721 (ester C=O), 1621 (amide C=O and quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.44 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.75 (d,  $J$  = 13.5 Hz, 1 H, *ortho* to F), 7.69 (d,  $J$  = 6.9 Hz, 1 H, CHNH), 7.43 (d,  $J$  = 7.6 Hz, 1 H, *meta* to F), 4.73 (br s, 1 H, CHOH), 3.77 - 3.81 (m, 1 H, CHOH), 3.74 - 3.77 (m, 1 H, CHNH), 3.73 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.24 (br. t,  $J$  = 4.2 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.55 (br t,  $J$  = 5.0 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.32 (t,  $J$  = 7.2 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.10 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.92 (dddd,  $J$  = 13.0, 8.7, 7.3, 6.0 Hz, 1 H, CHHCHNH), 1.77 (ddt,  $J$  = 12.6, 8.9, 6.3 Hz, 1 H, CHHCHOH), 1.68 (quin,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.53 - 1.64 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.42 (ddt,  $J$  = 12.9, 8.4, 5.2 Hz, 1 H, CHHCHOH), 1.31 (ddt,  $J$  = 13.0, 8.6, 6.4 Hz, 1 H, CHHCHNH), 1.22 - 1.28 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.06 - 1.12 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 171.9 (NH<sub>2</sub>C(=O)CH<sub>2</sub>), 171.5 (C(=O)CC(=O)OCH<sub>3</sub>), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 247.4 Hz, *ipso* to F), 148.2 (C=CC(=O)OCH<sub>3</sub>), 143.9 (d,  $J$  = 10.3 Hz, *ipso* to piperazine), 138.1 (*para* to F), 121.7 (d,  $J$  = 6.4 Hz, *para* to piperazine), 111.5 (d,  $J$  = 23.0 Hz, *ortho* to C=O and *ortho* to F), 109.0 (CC(=O)OCH<sub>3</sub>), 106.2 (*meta* to C=O and *meta* to F), 76.2 (CHOH), 57.6 (CHNH), 57.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 34.7 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.2 (C(=O)CH<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 22.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 20.6 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.5 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -125.5

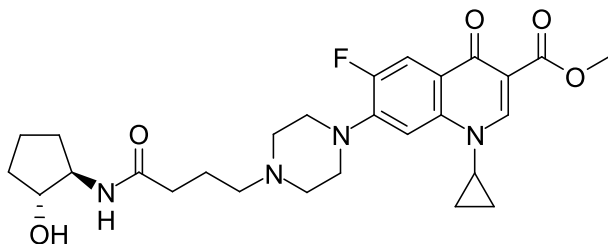
**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 515.2667, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>36</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 515.2670

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 8.0 ( $c$  / g(100 mL)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.



**9.54 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **179****



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **198** (200 mg, 0.367 mmol, 1 eq.), (1*R*,2*R*)-2-aminocyclopentan-1-ol **173** (80 mg, 0.791 mmol, 2.1 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (112 mg, 0.584 mmol, 1.6 eq.), 1-hydroxybenzotriazole (96 mg, 0.710 mmol, 1.9 eq.) and DIPEA (192  $\mu$ L, 142 mg, 1.10 mmol, 3 eq.) were dissolved in DMF (5 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of N<sub>2</sub> and the residue was purified by preparative HPLC (5-60% acetonitrile/water over 12 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The organic layer was removed and the aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2 $\times$ 10 mL). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **179** was obtained as a white amorphous solid (73.0 mg, 0.142 mmol, 39%).

**TLC**  $R_f$  = 0.43 (30% MeOH/EtOAc)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2973 (C-H), 2902 (C-H), 1728 (ester C=O), 1656 (amide C=O), 1613 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.44 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.75 (d,  $J$  = 13.5 Hz, 1 H, *ortho* to F), 7.70 (d,  $J$  = 7.2 Hz, 1 H, CHNH), 7.43 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.74 (d,  $J$  = 4.0 Hz, 1 H, CHOH), 3.78 - 3.82 (m, 1 H, CHOH), 3.74 - 3.78 (m, 1 H, CHNH), 3.74 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 7.2, 3.9 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.25 (t,  $J$  = 4.8 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.57 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.11 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.92 (dddd,  $J$  = 13.0, 8.7, 7.3, 6.0 Hz, 1 H, CHHCHNH), 1.78 (dddd,  $J$  = 12.6, 8.9, 6.3, 6.3 Hz, 1 H, CHHCHOH), 1.69 (quin,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.54 - 1.65 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.42 (ddt,  $J$  = 13.1, 8.2, 5.3 Hz, 1 H, CHHCHOH), 1.32 (dddd,  $J$  = 13.4, 8.5, 6.8, 5.8 Hz, 1 H, CHHCHNH), 1.21 - 1.29 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.07 - 1.13 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 171.9 (CH<sub>2</sub>C(=O)NH), 171.6 (C(=O)CC(=O)OCH<sub>3</sub>), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 246.5 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH<sub>3</sub>), 143.9 (d,  $J$  = 10.7 Hz, *ipso* to piperazine), 138.1 (*para* to F), 121.8 (d,  $J$  = 6.4 Hz, *para* to piperazine), 111.5 (d,  $J$  = 22.4 Hz, *ortho* to C=O and *ortho* to F), 109.0 (CC(=O)OCH<sub>3</sub>), 106.2 (*meta* to C=O and *meta* to F), 76.3 (CHOH), 57.6 (CHNH), 57.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 34.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.3 (C(=O)CH<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 22.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 20.6 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

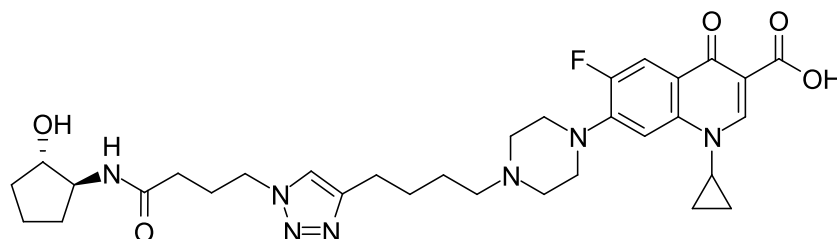
**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -124.3 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 515.2661, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>36</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 515.2670

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -6.0 ( $c$  / g(100 mL)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.

**9.55 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **180****



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (82.0 mg, 199 μmol, 4 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **176** (11.0 mg, 51.8 μmol, 1 eq.) were dissolved in 10% water/*t*-BuOH (3 mL), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (156 μl, 15.6 μmol, 0.3 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (312 μl, 31.2 μmol, 0.6 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 3 d. Water (10 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (10 mL) were added, then the organic layer was separated and dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (10 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **180** was obtained as a white amorphous solid (7.2 mg, 11.5 μmol, 22%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2955 (C-H), 2918 (C-H), 2850 (C-H), 1722 (carboxylic acid C=O), 1647 (amide C=O), 1627 (quinolone C=O) 1612 (triazole)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 15.22 (br s, 1 H, C(=O)OH), 8.67 (s, 1 H, *ortho* to C(=O)OH), 7.91 (d,  $J$  = 13.3 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H, CH=CCH<sub>2</sub>), 7.74 (d,  $J$  = 6.7 Hz, 1 H, CHNH), 7.56 (d,  $J$  = 7.4 Hz, 1 H, *meta* to F), 4.71 (d,  $J$  = 3.7 Hz, 1 H, CHOH), 4.29 (t,  $J$  = 6.6 Hz, 2 H, CH<sub>2</sub>NCH=C), 3.82 (tt,  $J$  = 6.5, 4.3 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.69 - 3.79 (m, 2 H, CHOH and CHNH), 3.30 - 3.34 (m, 6 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t,  $J$  = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 1.95 - 2.08 (m, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.89 (dddd,  $J$  = 12.8, 8.9, 7.4, 5.8 Hz, 1 H, CHHCHNH), 1.75 (ddt,  $J$  = 12.7, 9.0, 6.2 Hz, 1 H, CHHCHOH), 1.48 - 1.68 (m, 6 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub> and CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.40 (ddt,  $J$  = 13.0, 8.3, 5.3 Hz, 1 H, CHHCHOH), 1.28 - 1.35 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.24 - 1.31 (m, 1 H, CHHCHNH), 1.15 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (NHC(=O)CH<sub>2</sub>), 166.0 (C(=O)OH), 153.0 (d,  $J$  = 249.6 Hz, *ipso* to F), 148.1 (C=CC(=O)OH), 146.7 (CH=CCH<sub>2</sub>), 145.2 (d,  $J$  = 8.3 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 (NCH=CCH<sub>2</sub>), 118.7 (*para* to piperazine), 111.0 (d,  $J$  = 23.2 Hz, *ortho* to C=O and *ortho* to F), 106.7 (C(=O)OH), 106.5 (*meta* to C=O and *meta* to F), 76.2 (CHOH), 57.5

(CHNH), 57.4 (br s, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.3 (br s, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.3 (br s, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.8 (CH<sub>2</sub>NCH=CCH<sub>2</sub>), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 32.0 (C(=O)CH<sub>2</sub>), 29.4 (CH<sub>2</sub>CHNH), 26.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 20.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

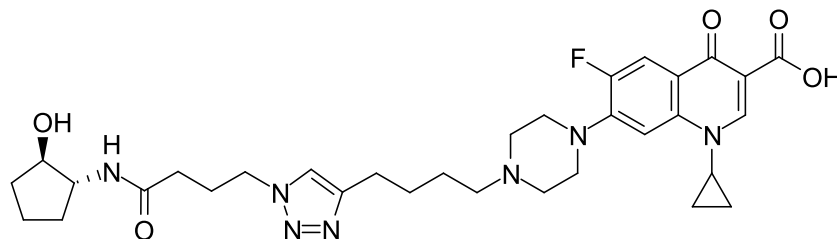
<sup>19</sup>F NMR (376.45 MHz, MeOD) δ / ppm = -121.5

HRMS (ESI<sup>+</sup>) *m/z* / Da = 624.3298, [M+H]<sup>+</sup> found, [C<sub>32</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 624.3310

[α]<sub>D</sub><sup>20</sup> / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -25.0 (*c* / g(100 mL)<sup>-1</sup> = 0.08, MeOH)

The compound has not been reported previously.

**9.56 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **181****



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (42.9 mg, 104 μmol, 1 eq.) and 4-azido-*N*-(((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **177** (22.0 mg, 104 μmol, 1 eq.) were dissolved in 10% water/*t*-BuOH (3 mL), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (104 μl, 10.4 μmol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208 μl, 20.8 μmol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (30 mL) were added, the organic layer was separated and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (4×30 mL). The combined organic layers were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (10 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **181** was obtained as a white amorphous solid (17.6 mg, 28.2 μmol, 27%).

IR (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2967 (C-H), 2902 (C-H), 1721 (carboxylic acid C=O), 1647 (amide C=O), 1627 (quinolone C=O), 1613 (triazole)

<sup>1</sup>H NMR (700 MHz, DMSO d<sub>6</sub>) δ / ppm = 8.64 (s, 1 H, *ortho* to C(=O)OH), 7.87 (d, *J* = 13.3 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H, CH=CCH<sub>2</sub>), 7.75 (d, *J* = 7.1 Hz, 1 H, CHNH), 7.54 (d, *J* = 7.5 Hz, 1 H, *meta* to F), 4.73 (d, *J* = 3.8 Hz, 1 H, CHOH), 4.29 (t, *J* = 6.9 Hz, 2 H, CH<sub>2</sub>NCH=C), 3.78 - 3.83 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.75 - 3.78 (m, 1 H, CHOH), 3.71 - 3.75 (m, 1 H, CHNH), 3.31 (br t, *J* = 4.3 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.63 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (br t, *J* = 4.2 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>), 2.37 (t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.03 - 2.06 (m, 2 H, C(=O)CH<sub>2</sub>), 1.97 - 2.02 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.89 (dddd, *J* =

13.1, 8.9, 7.4, 5.7 Hz, 1 H,  $\text{CHHCHNH}$ ), 1.75 (ddt,  $J = 13.0, 8.9, 6.4, 6.4$  Hz, 1 H,  $\text{CHHCHOH}$ ), 1.61 - 1.66 (m, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 1.57 - 1.61 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.54 - 1.57 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.49 - 1.53 (m, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 1.40 (ddt,  $J = 13.0, 8.4, 5.3, 5.3$  Hz, 1 H,  $\text{CHHCHOH}$ ), 1.29 - 1.32 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.25 - 1.29 (m, 1 H,  $\text{CHHCHNH}$ ), 1.13 - 1.20 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

$^{13}\text{C}$  NMR (175 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 176.3 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$ ), 170.9 ( $\text{NHC}(=\text{O})\text{CH}_2$ ), 166.1 ( $\text{C}(=\text{O})\text{OH}$ ), 153.0 (d,  $J = 251.4$  Hz, *ipso* to F), 147.9 ( $\text{C}=\text{CC}(=\text{O})\text{OH}$ ), 146.9 ( $\text{CH}=\text{CCH}_2$ ), 145.2 (d,  $J = 8.7$  Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 ( $\text{NCH}=\text{CCH}_2$ ), 118.7 (d,  $J = 5.8$  Hz, *para* to piperazine), 111.0 (d,  $J = 23.3$  Hz, *ortho* to C=O and *ortho* to F), 106.3 (*meta* to C=O and *meta* to F and  $\text{CC}(=\text{O})\text{OH}$ ), 76.2 ( $\text{CHOH}$ ), 57.6 ( $\text{CHNH}$ ), 57.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 52.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.5 (d,  $J = 4.4$  Hz,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 48.8 ( $\text{CH}_2\text{NCH}=\text{CCH}_2$ ), 35.8 ( $\text{NCH}(\text{CH}_2)_2$ ), 32.2 ( $\text{CH}_2\text{CHOH}$ ), 32.0 ( $\text{C}(=\text{O})\text{CH}_2$ ), 29.5 ( $\text{CH}_2\text{CHNH}$ ), 26.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 26.0 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 25.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 25.0 ( $\text{CH}=\text{CCH}_2$ ), 20.5 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 7.6 ( $\text{NCH}(\text{CH}_2)_2$ )

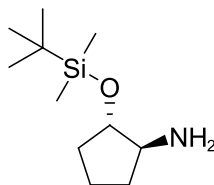
$^{19}\text{F}$  NMR (376.45 MHz, MeOD)  $\delta$  / ppm = -122.1 (s, ciprofloxacin F)

HRMS (ESI<sup>+</sup>)  $m/z$  / Da = 624.3314,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{32}\text{H}_{43}\text{FN}_7\text{O}_5]^+$  requires 624.3310

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = -3.6 ( $c$  /  $\text{g}(100\text{ mL})^{-1}$  = 0.0833, MeOH)

The compound has not been reported previously.

## 9.57 (1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine **182**



(1*S*,2*S*)-2-Aminocyclopentan-1-ol **172** (0.480 g, 4.75 mmol) was stirred in dry  $\text{CH}_2\text{Cl}_2$  (20 mL) under  $\text{N}_2$  at 0  $^{\circ}\text{C}$ . TEA (3.14 mL, 2.28 g, 22.5 mmol, 5 eq.) was added dropwise, followed by TBDMSOTf (3 mL, 3.45 g, 13.1 mmol, 3 eq.) dropwise. The reaction was allowed to reach r.t. and stirred for 1 h. The reaction was washed with water (20 mL) and the organic phase dried with  $\text{Na}_2\text{SO}_4$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ , 4% MeOH/ $\text{CH}_2\text{Cl}_2$ ). **182** was obtained as a yellow oil (1.00 g, 4.64 mmol, 98%).

TLC  $R_f$  = 0.23 (10% MeOH/ $\text{CH}_2\text{Cl}_2$ )

IR (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2954 (C-H), 2931 (C-H), 2888 (C-H), 2859 (C-H), 1625 (N-H bend)

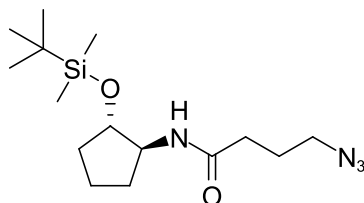
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 4.13 (q,  $J = 5.8$  Hz, 1 H,  $\text{CHOSi}$ ), 3.31 (td,  $J = 7.1, 5.2$  Hz, 1 H,  $\text{CHNH}_2$ ), 2.09 - 2.19 (m, 1 H,  $\text{CHHCHNH}_2$ ), 1.97 (ddq,  $J = 8.8, 7.0, 6.0$  Hz, 1 H,  $\text{CHHCHOSi}$ ), 1.74 - 1.86 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CHOSi}$ ), 1.64 - 1.74 (m, 1 H,  $\text{CHHCHOSi}$ ), 1.58 (ddt,  $J = 13.2, 9.1, 6.0$  Hz, 1 H,  $\text{CHHCHNH}_2$ ), 0.88 (s, 9 H,  $\text{C}(\text{CH}_3)_3$ ), 0.09 (s, 3 H,  $\text{SiCH}_3$ ), 0.07 (s, 3 H,  $\text{SiCH}_3$ )

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 76.3 ( $\text{CHOSi}$ ), 59.7 ( $\text{CHNH}$ ), 32.2 ( $\text{CH}_2\text{CHOSi}$ ), 26.8 ( $\text{CH}_2\text{CHNH}_2$ ), 25.6 ( $\text{C}(\text{CH}_3)_3$ ), 19.7 ( $\text{CH}_2\text{CH}_2\text{CHOSi}$ ), 17.7 ( $\text{C}(\text{CH}_3)_3$ ), -4.8 ( $\text{SiCH}_3$ ), -5.2 ( $\text{SiCH}_3$ )

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 216.1785, [M+H]<sup>+</sup> found, [C<sub>11</sub>H<sub>26</sub>NOSi]<sup>+</sup> requires 216.1784

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 40.0 ( $c$  / g(100 mL)<sup>-1</sup> = 0.05, MeOH) The compound has not been reported previously.

### 9.58 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide 186



(1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine **182** (50 mg, 0.232 mmol, 1 eq.) and NaHCO<sub>3</sub> (22.0 mg, 0.262 mmol, 1.1 eq.) were added to CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and water (3 mL) at 0 °C, and 4-bromobutyryl chloride (25.3 mL, 40.5 mg, 0.219 mmol, 0.95 eq.) was added dropwise. The mixture was stirred for 3 h at 0 °C. The aqueous layer was removed and NaN<sub>3</sub> (100 mg, 1.54 mmol, 6.6 eq.) and DMF (3 mL) were added. The mixture was then stirred at 40 °C for 6 h. The solvents were then evaporated using a N<sub>2</sub> stream and the residue was purified by column chromatography (SiO<sub>2</sub>, 1% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **186** was obtained as a clear liquid (71 mg, 0.217 mmol, 99%).

**TLC**  $R_f$  = 0.84 (1% MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3288 (N-H), 2953 (C-H), 2933 (C-H), 2883 (C-H), 2857 (C-H), 2095 (azide), 1639 (amide C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 5.35 (d,  $J$  = 5.1 Hz, 1 H, NH), 3.97 - 4.01 (m, 1 H, CHOSi), 3.93 - 3.98 (m, 1 H, CHNH), 3.35 (t,  $J$  = 6.6 Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 2.24 (t,  $J$  = 7.0 Hz, 2 H, CH<sub>2</sub>C=O), 2.09 - 2.19 (m, 1 H, CHHCHNH), 1.89 - 1.97 (quin,  $J$  = 6.8 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.74 - 1.84 (m, 2 H, CHHCHOSi and CHHCH<sub>2</sub>CHOSi), 1.60 - 1.70 (m, 1 H, CHHCH<sub>2</sub>CHOSi), 1.51 - 1.61 (m, 1 H, CHHCHOSi), 1.31 - 1.39 (m, 1 H, CHHCHNH), 0.87 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 3 H, SiCH<sub>3</sub>), 0.06 (s, 3 H, SiCH<sub>3</sub>)

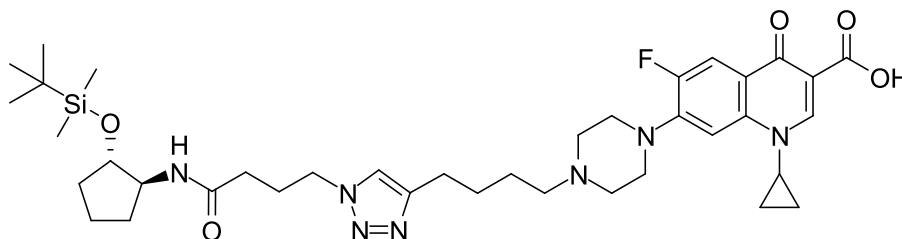
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 171.17 (C=O), 77.80 (CHOSi), 58.36 (CHNH), 50.77 (CH<sub>2</sub>N<sub>3</sub>), 33.29 (CH<sub>2</sub>C=O), 32.57 (CH<sub>2</sub>CHOSi), 29.36 (CH<sub>2</sub>CHNH), 25.72 (C(CH<sub>3</sub>)<sub>3</sub>), 24.77 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.40 (CH<sub>2</sub>CH<sub>2</sub>CHOSi), 17.95 (C(CH<sub>3</sub>)<sub>3</sub>), -4.75 (SiCH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 327.2221, [M+H]<sup>+</sup> found, [C<sub>15</sub>H<sub>31</sub>N<sub>4</sub>O<sub>2</sub>Si]<sup>+</sup> requires 327.2216

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 12.4 ( $c$  / g(100 mL)<sup>-1</sup> = 0.5, MeOH)

The compound has not been reported previously.

**9.59 7-(4-(4-(1-(4-(((1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **190****



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (42.9 mg, 104  $\mu$ mol, 1 eq.) and 4-azido-*N*-(((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide **186** (33.9 mg, 104  $\mu$ mol, 1 eq.) were dissolved in 10% water/*t*-BuOH (3 mL), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (104  $\mu$ l, 10.4  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208  $\mu$ l, 20.8  $\mu$ mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 16 h, then solvent was removed under reduced pressure. The residue was partitioned between water (10 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL), the organic layer was separated and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The combined organic layers were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **190** was obtained as a clear amorphous solid (67.1 mg, 90.9  $\mu$ mol, 87%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2951 (C-H), 2929 (C-H), 2856 (C-H), 1741 (carboxylic acid C=O), 1640 (amide C=O), 1627 (quinolone C=O), 1612 (triazole)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 8.67 (s, 1 H, *ortho* to C(=O)OH), 7.87 (d, *J* = 13.1 Hz, 1 H, *ortho* to F), 7.34 (s, 1 H, CH=CCH<sub>2</sub>), 7.33 (d, *J* = 8.2 Hz, 1 H, *meta* to F), 5.92 (t, *J* = 6.6 Hz, 1 H, CHNH), 4.35 (t, *J* = 6.7 Hz, 2 H, CH<sub>2</sub>NCH=C), 3.96 - 4.02 (m, 1 H, CHOSi), 3.90 - 3.96 (m, 1 H, CHNH), 3.55 (tt, *J* = 6.7, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.34 (br t, *J* = 5.0 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.71 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.66 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.46 (t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.03 - 2.22 (m, 5 H, CHHCHNH, C(=O)CH<sub>2</sub> and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.65 - 1.83 (m, 4 H, CHHCHOSi, CHHCH<sub>2</sub>CHOSi and NCH=CCH<sub>2</sub>CH<sub>2</sub>), 1.47 - 1.65 (m, 4 H, CHHCHOSi, CHHCH<sub>2</sub>CHOSi and NCH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.33 - 1.41 (m, 3 H, CHHCHNH and NCH(CH<sub>2</sub>)<sub>2</sub>), 1.14 - 1.20 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 0.82 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.03 (s, 3 H, SiCH<sub>3</sub>), 0.01 (s, 3 H, SiCH<sub>3</sub>)

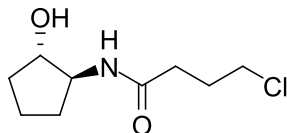
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 176.9 (C(=O)CC(=O)OH), 170.9 (CH<sub>2</sub>C(=O)NH), 166.9 (C(=O)OH), 153.5 (d, *J* = 251.4 Hz, *ipso* to F), 147.9 (CH=CCH<sub>2</sub>), 147.2 (C=CC(=O)OH), 145.8 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.0 (*para* to F), 120.9 (NCH=CCH<sub>2</sub>), 119.4 (d, *J* = 7.8 Hz, *para* to piperazine), 112.0 (d, *J* = 23.4 Hz, *ortho* to C=O and *ortho* to F), 107.7 (CC(=O)OH), 104.7 (d, *J* = 3.5 Hz, *meta* to C=O and *meta* to F), 77.7 (CHOSi), 58.2 (CHNH), 57.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (d, *J* = 6.1 Hz, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.9 (d, *J* = 3.5 Hz, CH<sub>2</sub>NCH=CCH<sub>2</sub>), 35.3 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.6 (C(=O)CH<sub>2</sub>), 32.6 (CH<sub>2</sub>CHOSi), 29.3 (CH<sub>2</sub>CHNH), 27.2 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.0 - 26.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.6 (C(CH<sub>3</sub>)<sub>3</sub>), 25.4 (CH=CCH<sub>2</sub>), 20.4 (CH<sub>2</sub>CH<sub>2</sub>CHOSi), 17.8 (C(CH<sub>3</sub>)<sub>3</sub>), 8.1 (NCH(CH<sub>2</sub>)<sub>2</sub>), -4.8 (SiCH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 738.4164, [M+H]<sup>+</sup> found, [C<sub>38</sub>H<sub>57</sub>FN<sub>7</sub>O<sub>5</sub>Si]<sup>+</sup> requires 738.4169

$$[\alpha]_D^{20} / ^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = 4.5 \text{ (} c / \text{g(100 mL)}^{-1} = 0.2, \text{ MeOH)}$$

The compound has not been reported previously.

### 9.60 4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **193**



(1*S*,2*S*)-2-Aminocyclopentan-1-ol **172** (72.3 mg, 716  $\mu\text{mol}$ , 1 eq.), TEA (500  $\mu\text{l}$ , 363 mg, 3.58 mmol, 5 eq.) and  $\text{CH}_2\text{Cl}_2$  (5 mL) were stirred at 0  $^\circ\text{C}$ , and 4-chlorobutyryl chloride **192** (179  $\mu\text{l}$ , 226 mg, 1.60 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0  $^\circ\text{C}$  for 30 min, then water (10 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with 10% *i*-PrOH/ $\text{CHCl}_3$  (2 $\times$ 10 mL). The combined organic layers were dried with  $\text{MgSO}_4$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ ,  $\text{Et}_2\text{O}$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **193** was obtained as a white amorphous solid (35.6 mg, 173  $\mu\text{mol}$ , 24%).

**TLC**  $R_f$  = 0.35 (EtOAc)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 6.05 (br s, 1 H,  $\text{NH}$ ), 4.55 (br s, 1 H,  $\text{OH}$ ), 3.95 (q,  $J$  = 6.6 Hz, 1 H,  $\text{CHOH}$ ), 3.82 (tt,  $J$  = 8.4, 5.3 Hz, 1 H,  $\text{CHNH}$ ), 3.60 (t,  $J$  = 6.2 Hz, 2 H,  $\text{CH}_2\text{Cl}$ ), 2.38 (t,  $J$  = 7.0 Hz, 2 H,  $\text{CH}_2\text{C=O}$ ), 2.05 - 2.17 (m, 3 H,  $\text{CHHCHNH}$  and  $\text{CH}_2\text{CH}_2\text{Cl}$ ), 1.94 - 2.05 (m, 1 H,  $\text{CHHCHOH}$ ), 1.74 - 1.86 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.58 - 1.74 (m, 2 H,  $\text{CHHCH}_2\text{CHOH}$  and  $\text{CHHCHOH}$ ), 1.42 (dq,  $J$  = 12.5, 8.4 Hz, 1 H,  $\text{CHHCHNH}$ )

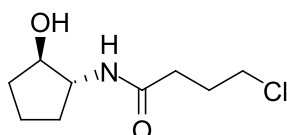
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 173.8 ( $\text{C=O}$ ), 79.4 ( $\text{CHOH}$ ), 60.6 ( $\text{CHNH}$ ), 44.4 ( $\text{CH}_2\text{Cl}$ ), 32.8 ( $\text{CH}_2\text{C=O}$ ), 32.4 ( $\text{CH}_2\text{CHOH}$ ), 30.2 ( $\text{CH}_2\text{CHNH}$ ), 28.0 ( $\text{CH}_2\text{CH}_2\text{Cl}$ ), 21.2 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 206.0939,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_9\text{H}_{17}\text{ClNO}_2]^+$  requires 206.0948

$$[\alpha]_D^{20} / ^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = 10.0 \text{ (} c / \text{g(100 mL)}^{-1} = 0.05, \text{ MeOH)}$$

The compound has not been reported previously.

### 9.61 4-Chloro-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **194**



(1*R*,2*R*)-2-Aminocyclopentan-1-ol **173** (500 mg, 4.94 mmol, 1 eq.), TEA (827  $\mu\text{l}$ , 600 mg, 5.93 mmol, 1.2 eq.) and  $\text{CH}_2\text{Cl}_2$  (20 mL) were stirred at 0  $^\circ\text{C}$  and 4-chlorobutyryl chloride **192** (608  $\mu\text{l}$ , 766 mg, 5.43 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0  $^\circ\text{C}$  for 30 min, then water (50 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (7 $\times$ 50 mL). The

combined organic layers were dried with  $\text{MgSO}_4$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ ,  $\text{Et}_2\text{O}$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **194** was obtained as a white amorphous solid (651 mg, 3.16 mmol, 64%).

**TLC**  $R_f = 0.35$  ( $\text{EtOAc}$ )

**IR** (neat)  $\nu_{\max} / \text{cm}^{-1} = 3278$  (N-H and O-H), 2962 (C-H), 2876 (C-H), 1636 (amide C=O)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 6.12$  (br s, 1 H,  $\text{NH}$ ), 4.42 (br s, 1 H,  $\text{OH}$ ), 3.94 (q,  $J = 6.6$  Hz, 1 H,  $\text{CHOH}$ ), 3.82 (tt,  $J = 8.4, 5.3$  Hz, 1 H,  $\text{CHNH}$ ), 3.60 (t,  $J = 6.2$  Hz, 2 H,  $\text{CH}_2\text{Cl}$ ), 2.38 (t,  $J = 7.2$  Hz, 2 H,  $\text{CH}_2\text{C=O}$ ), 2.05 - 2.16 (m, 3 H,  $\text{CHHCHNH}$  and  $\text{CH}_2\text{CH}_2\text{Cl}$ ), 1.96 - 2.04 (m, 1 H,  $\text{CHHCHOH}$ ), 1.74 - 1.85 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.58 - 1.73 (m, 2 H,  $\text{CHHCH}_2\text{CHOH}$  and  $\text{CHHCHOH}$ ), 1.43 (dq,  $J = 12.7, 8.3$  Hz, 1 H,  $\text{CHHCHNH}$ )

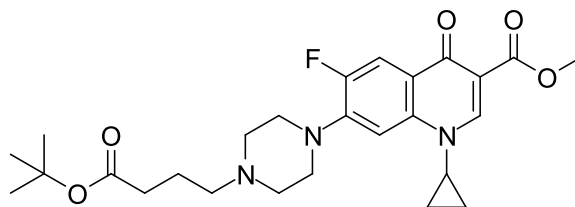
**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 173.8$  ( $\text{C=O}$ ), 79.4 ( $\text{CHOH}$ ), 60.6 ( $\text{CHNH}$ ), 44.4 ( $\text{CH}_2\text{Cl}$ ), 32.8 ( $\text{CH}_2\text{C=O}$ ), 32.4 ( $\text{CH}_2\text{CHOH}$ ), 30.1 ( $\text{CH}_2\text{CHNH}$ ), 28.0 ( $\text{CH}_2\text{CH}_2\text{Cl}$ ), 21.1 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

**HRMS** ( $\text{ESI}^+$ )  $m/z / \text{Da} = 228.0787$ ,  $[\text{M}+\text{Na}]^+$  found,  $[\text{C}_9\text{H}_{16}\text{ClNNaO}_2]^+$  requires 228.0762

$[\alpha]_D^{20} / ^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = -13.0$  ( $c / \text{g}(100 \text{ mL})^{-1} = 0.5$ ,  $\text{MeOH}$ )

The compound has not been reported previously.

## 9.62 Methyl 7-(4-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **197**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **151** (200 mg, 0.579 mmol, 1 eq.), *tert*-butyl 4-bromobutanoate **196** (103  $\mu\text{l}$ , 130 mg, 0.581 mmol, 1 eq.),  $\text{NaI}$  (86.9 mg, 0.580 mmol, 1 eq.),  $\text{TEA}$  (316  $\mu\text{l}$ , 229 mg, 2.27 mmol, 4 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at  $100^\circ\text{C}$  for 8 h. A second portion of *tert*-butyl 4-bromobutanoate **222** (103  $\mu\text{l}$ , 130 mg, 0.581 mmol, 1 eq.) was added, and the mixture was stirred in the microwave reactor at  $100^\circ\text{C}$  for a further 8 h. The mixture was then dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography ( $\text{SiO}_2$ , 0-4%  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ). **197** was obtained as a white amorphous solid (141 mg, 0.289 mmol, 50%).

**TLC**  $R_f = 0.12$  (4%  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\max} / \text{cm}^{-1} = 2962$  (C-H), 2831 (C-H), 1732 (*t*-Bu ester C=O) 1717 (ciprofloxacin ester C=O), 1621 (quinolone C=O)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 8.39$  (s, 1 H, *ortho* to  $\text{C(=O)OCH}_3$ ), 7.82 (d,  $J = 13.3$  Hz, 1 H, *ortho*



to F), 7.17 (d,  $J = 7.2$  Hz, 1 H, *meta* to F), 3.83 (s, 3 H,  $\text{CH}_3$ ), 3.40 (tt,  $J = 7.2, 3.6$  Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.22 (t,  $J = 4.3$  Hz, 4 H,  $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 2.63 (t,  $J = 4.4$  Hz, 4 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.41 (t,  $J = 7.3$  Hz, 2 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.25 (t,  $J = 7.4$  Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 1.78 (quin,  $J = 7.3$  Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 1.41 (s, 9 H,  $\text{C}((\text{CH}_3)_3)$ ), 1.24 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.09 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

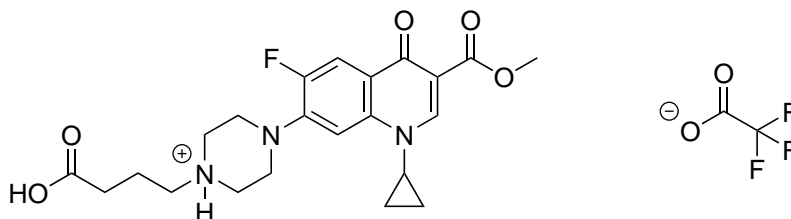
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 172.7 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OCH}_3$ ), 172.6 ( $\text{C}(=\text{O})\text{OC}(\text{CH}_3)_3$ ), 165.9 ( $\text{C}(=\text{O})\text{OCH}_3$ ), 153.1 (d,  $J = 249.7$  Hz, *ipso* to F), 148.1 ( $\text{C}=\text{CC}(=\text{O})\text{OCH}_3$ ), 144.3 (d,  $J = 10.4$  Hz, *ipso* to piperazine), 137.7 (*para* to F), 122.5 (d,  $J = 6.9$  Hz, *para* to piperazine), 112.6 (d,  $J = 22.5$  Hz, *ortho* to  $\text{C}=\text{O}$  and *ortho* to F), 109.5 ( $\text{CC}(=\text{O})\text{OCH}_3$ ), 104.7 (*meta* to  $\text{C}=\text{O}$  and *meta* to F), 80.0 ( $\text{C}(\text{CH}_3)_3$ ), 57.4 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 52.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 51.7 ( $\text{CH}_3$ ), 49.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 34.4 ( $\text{NCH}(\text{CH}_2)_2$ ), 33.2 ( $\text{C}(=\text{O})\text{CH}_2$ ), 28.0 ( $\text{C}(\text{CH}_3)_3$ ), 22.0 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 7.9 ( $\text{NCH}(\text{CH}_2)_2$ )

$^{19}\text{F}$  NMR (376.45 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = -123.5 (s, ciprofloxacin F)

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 488.2562,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{26}\text{H}_{35}\text{FN}_3\text{O}_5]^+$  requires 488.2561

The compound has not been reported previously.

### 9.63 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **198**



Methyl 7-(4-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **197** (20 mg, 41.0  $\mu\text{mol}$ ) and TFA (0.2 mL) were stirred in  $\text{CH}_2\text{Cl}_2$  (1.8 mL) at r.t. for 16 h then evaporated under reduced pressure. **198** was obtained as a white solid (21.4 mg, 39.2  $\mu\text{mol}$ , 96%).

**mp**  $T$  /  $^\circ\text{C}$  = 225-231 ( $\text{CH}_2\text{Cl}_2$ , decomposes)

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 1723 (ciprofloxacin ester  $\text{C}=\text{O}$ ), 1699 (alkyl carboxylic acid  $\text{C}=\text{O}$ ), 1673 (TFA  $\text{C}=\text{O}$ ), 1615 (quinolone  $\text{C}=\text{O}$ )

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 8.47 (s, 1 H, *ortho* to  $\text{C}(=\text{O})\text{OH}$ ), 7.80 (d,  $J = 13.2$  Hz, 1 H, *ortho* to F), 7.47 (d,  $J = 7.4$  Hz, 1 H, *meta* to F), 3.73 (s, 3 H,  $\text{CH}_3$ ), 3.66 (tt,  $J = 7.2, 3.7$  Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.30 - 3.54 (br s, 8 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$  and  $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 3.13 - 3.22 (m, 2 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.36 (t,  $J = 7.1$  Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 1.87 - 1.98 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 1.22 - 1.30 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.06 - 1.15 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

$^{13}\text{C}$  NMR (101 MHz,  $\text{DMSO}-d_6$ )  $\delta$  / ppm = 173.5 ( $\text{CH}_2\text{C}(=\text{O})\text{OH}$ ), 171.6 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OCH}_3$ ), 164.9 ( $\text{C}(=\text{O})\text{OCH}_3$ ), 158.2 (q,  $J = 31.5$  Hz,  $\text{CF}_3\text{C}(=\text{O})\text{OH}$ ), 152.5 (d,  $J = 247.6$  Hz, *ipso* to F), 148.5 ( $\text{C}=\text{CC}(=\text{O})\text{OH}$ ), 142.3 (d,  $J = 10.7$  Hz, *ipso* to piperazine), 138.0 (*para* to F), 122.6 (d,  $J = 6.4$  Hz, *para* to piperazine), 117.2 (q,

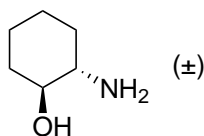
$J = 299.8$  Hz,  $\underline{\text{C}}\text{F}_3$ ), 111.9 (d,  $J = 22.4$  Hz, *ortho* to C=O and *ortho* to F), 109.1 ( $\underline{\text{C}}\text{C}(=\text{O})\text{OCH}_3$ ), 106.9 (*meta* to C=O and *meta* to F), 55.1 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\underline{\text{C}}\text{H}_2\text{N}$ ), 51.4 ( $\underline{\text{C}}\text{H}_3$ ), 50.8 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{C}}\text{H}_2)\underline{\text{C}}\text{H}_2$ ), 46.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\underline{\text{C}}\text{H}_2)\text{CH}_2\text{CH}_2$ ), 46.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\underline{\text{C}}\text{H}_2$ ), 34.9 ( $\text{NCH}(\underline{\text{C}}\text{H}_2)_2$ ), 30.6 ( $\text{C}(=\text{O})\underline{\text{C}}\text{H}_2$ ), 19.1 ( $\text{C}(=\text{O})\text{CH}_2\underline{\text{C}}\text{H}_2$ ), 7.6 ( $\text{NCH}(\underline{\text{C}}\text{H}_2)_2$ )

$^{19}\text{F}$  NMR (376.45 MHz, DMSO  $\text{d}_6$ )  $\delta$  / ppm = -73.6 (s,  $\underline{\text{C}}\text{F}_3$ ), -124.6 (s, ciprofloxacin F)

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 432.1921,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{22}\text{H}_{27}\text{FN}_3\text{O}_5]^+$  requires 432.1935

The compound has not been reported previously.

## 9.64 (*trans*)-2-Aminocyclohexan-1-ol **200**



Cyclohexene oxide **199** (10 mL, 9.70 g, 98.8 mmol, 1 eq.),  $\text{NH}_3$  (90 mL, 35% w/w aq., 27.7 g, 791 mmol, 8 eq.) and MeOH (100 mL) were stirred at r.t. for 72 h. The solvent was removed by blowing a stream of  $\text{N}_2$  over it, followed by evaporation under high vacuum. **200** was obtained as a white amorphous solid (9.90 g, 85.2 mmol, 86%)

TLC  $R_f = 0.04$  (30% MeOH/ $\text{CH}_2\text{Cl}_2$ )

IR (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 3350 (N-H), 3306 (br, O-H), 2927 (C-H), 2853 (C-H)

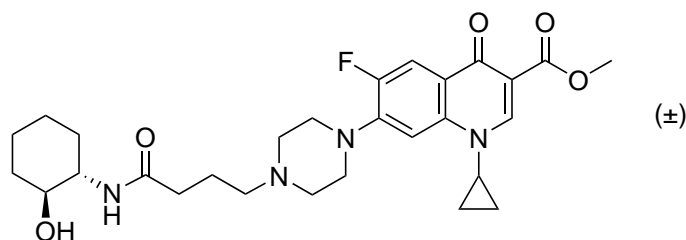
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 3.01 (td,  $J = 9.4, 4.8$  Hz, 1 H,  $\underline{\text{C}}\text{HOH}$ ), 2.80 - 2.92 (m, 2 H,  $\text{OH}$  and  $\text{NH}_2$ ), 2.35 (ddd,  $J = 11.1, 9.1, 4.1$  Hz, 1 H,  $\underline{\text{C}}\text{HNH}_2$ ), 1.77 - 1.84 (m, 1 H,  $\underline{\text{C}}\text{HHCHOH}$ ), 1.69 - 1.76 (m, 1 H,  $\underline{\text{C}}\text{HHCHNH}_2$ ), 1.56 - 1.66 (m, 1 H,  $\underline{\text{C}}\text{HHCH}_2\text{CHOH}$ ), 1.45 - 1.56 (m, 1 H,  $\underline{\text{C}}\text{HHCH}_2\text{CHNH}_2$ ), 1.07 - 1.19 (m, 3 H,  $\underline{\text{C}}\text{HHCH}_2\text{CHOH}$ ,  $\underline{\text{C}}\text{HHCH}_2\text{CHNH}_2$  and  $\underline{\text{C}}\text{HHCHOH}$ ), 0.94 - 1.05 (m, 1 H,  $\underline{\text{C}}\text{HHCHNH}_2$ )

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 75.4 ( $\underline{\text{C}}\text{HOH}$ ), 56.6 ( $\underline{\text{C}}\text{HNH}_2$ ), 33.8 ( $\underline{\text{C}}\text{H}_2\text{CHOH}$  and  $\underline{\text{C}}\text{H}_2\text{CHNH}_2$ ), 24.7 ( $\underline{\text{C}}\text{H}_2\text{CH}_2\text{CHNH}_2$ ), 24.6 ( $\underline{\text{C}}\text{H}_2\text{CH}_2\text{CHOH}$ )

HRMS ( $\text{ESI}^+$ )  $m/z$  / Da = 116.1070,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_6\text{H}_{14}\text{NO}]^+$  requires 116.1070

The data are consistent with the literature.<sup>228</sup>

**9.65 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **201****



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **198** (200 mg, 0.367 mmol, 1 eq.), (*trans*)-2-aminocyclohexan-1-ol **200** (91.1 mg, 0.791 mmol, 2.1 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (112 mg, 0.584 mmol, 1.6 eq.), 1-hydroxybenzotriazole (96 mg, 0.710 mmol, 1.9 eq.) and DIPEA (192  $\mu$ L, 142 mg, 1.10 mmol, 3 eq.) were dissolved in DMF (5 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of N<sub>2</sub> and the residue was purified by preparative HPLC (5-50% acetonitrile/water over 10 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **201** was obtained as a white amorphous solid (61.2 mg, 0.116 mmol, 32%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3303 (N-H), 2930 (C-H), 2851 (C-H), 2833 (C-H), 1698 (ester C=O), 1646 (amide C=O), 1614 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 8.60 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.79 (d,  $J$  = 13.5 Hz, 1 H, *ortho* to F), 7.46 (d,  $J$  = 7.2 Hz, 1 H, *meta* to F), 3.84 (s, 3 H, CH<sub>3</sub>), 3.62 - 3.68 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.58 (td,  $J$  = 10.3, 4.2 Hz, 1 H, CHNH), 3.38 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.32 - 3.36 (m, 1 H, CHOH), 2.83 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.60 (t,  $J$  = 7.3 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.32 (td,  $J$  = 7.1, 3.1 Hz, 2 H, C(=O)CH<sub>2</sub>), 1.96 - 2.04 (m, 1 H, CHHCHOH), 1.87 - 1.96 (m, 3 H, CHHCHNH and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.72 - 1.77 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.66 - 1.72 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.25 - 1.39 (m, 5 H, CHHCHOH, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH and NCH(CHH)<sub>2</sub>), 1.15 - 1.25 (m, 3 H, CHHCHOH and NCH(CHH)<sub>2</sub>)

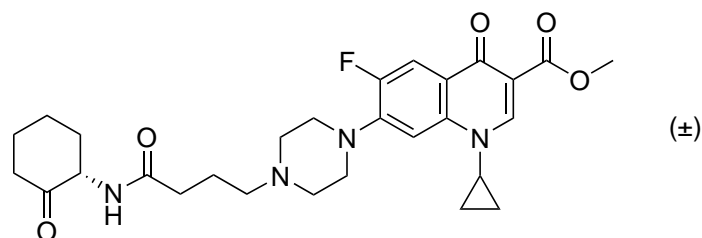
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.8 (CH<sub>2</sub>C(=O)NH), 175.3 (C(=O)CC(=O)OCH<sub>3</sub>), 166.8 (C(=O)OCH<sub>3</sub>), 154.9 (d,  $J$  = 248.8 Hz, *ipso* to F), 150.2 (C=CC(=O)OCH<sub>3</sub>), 146.1 (d,  $J$  = 10.8 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.5 (d,  $J$  = 7.5 Hz, *para* to piperazine), 113.2 (d,  $J$  = 23.2 Hz, *ortho* to C=O and *ortho* to F), 110.2 (CC(=O)OCH<sub>3</sub>), 107.2 (*meta* to C=O and *meta* to F), 74.1 (CHOH), 58.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 56.4 (CHNH), 54.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.3 (CH<sub>3</sub>), 50.5 (d,  $J$  = 5.0 Hz, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.4 (NCH(CH<sub>2</sub>)<sub>2</sub>), 35.7 (CH<sub>2</sub>CHOH), 35.1 (C(=O)CH<sub>2</sub>), 32.8 (CH<sub>2</sub>CHNH), 25.9 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 23.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 8.7 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.7 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 529.2827, [M+H]<sup>+</sup> found, [C<sub>28</sub>H<sub>38</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 529.2826

The compound has not been reported previously.

**9.66 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **202****



Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **201** (5.2 mg, 9.84  $\mu$ mol, 1 eq.) and Dess-Martin periodinane (16.4 mg, 38.7  $\mu$ mol, 4 eq.) were stirred in  $\text{CH}_2\text{Cl}_2$  (3 mL) at r.t. for 6 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure to a volume of 20 mL, then  $\text{NaHCO}_3$  (aq., sat., 30 mL) and 10% *i*-PrOH/ $\text{CHCl}_3$  (30 mL) were added. The organic layer was dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **202** was obtained as a white amorphous solid (3.6 mg, 6.8  $\mu$ mol, 69%).

**TLC**  $R_f$  = 0.74 (30% MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  = 2921 (C-H), 2852 (C-H), 1721 (ketone C=O), 1698 (ester C=O), 1639 (amide C=O), 1620 (quinolone C=O)

**$^1\text{H}$  NMR** (400 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 8.45 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.87 (d,  $J$  = 6.2 Hz, 1 H, NH), 7.76 (d,  $J$  = 13.4 Hz, 1 H, *ortho* to F), 7.44 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.42 (dddd,  $J$  = 13.0, 7.6, 6.0, 1.0 Hz, 1 H, CHNH), 3.73 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 7.1, 3.9 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.25 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.58 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.45 - 2.53 (m, 1 H, CHHC(=O)CHNH), 2.36 (br s, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.26 (dt,  $J$  = 13.4, 2.6, 1.6 Hz, 1 H, CHHC(=O)CHNH), 2.16 - 2.22 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.12 (ddq,  $J$  = 12.7, 6.0, 2.8 Hz, 1 H, CHHCHNH), 2.00 (ddquin,  $J$  = 13.2, 6.0, 2.9 Hz, 1 H, CHHCH<sub>2</sub>C(=O)), 1.65 - 1.83 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.41 - 1.56 (m, 2 H, CHHCHNH and CHHCH<sub>2</sub>C(=O)), 1.20 - 1.30 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.05 - 1.13 (m, 2 H, NCH(CHH)<sub>2</sub>)

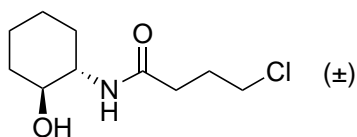
**$^{13}\text{C}$  NMR** (101 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 207.5 (C(=O)CHNH), 171.7 (C(=O)CC(=O)OCH<sub>3</sub>), 171.6 (CH<sub>2</sub>C(=O)NH), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 247.6 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH<sub>3</sub>), 143.9 (br s, *ipso* to piperazine), 138.1 (*para* to F), 121.8 (d,  $J$  = 6.4 Hz, *para* to piperazine), 111.5 (d,  $J$  = 22.4 Hz, *ortho* to C=O and *ortho* to F), 109.0 (CC(=O)OCH<sub>3</sub>), 106.3 (*meta* to C=O and *meta* to F), 57.0 (CHNH and C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.3 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.5 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 40.6 (CH<sub>2</sub>C(=O)CHNH), 34.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.9 (CH<sub>2</sub>CHNH), 32.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 27.2 (CH<sub>2</sub>CH<sub>2</sub>C(=O)CHNH), 23.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 22.4 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**$^{19}\text{F}$  NMR** (376.45 MHz, DMSO  $d_6$ )  $\delta$  / ppm = -124.3 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 527.2654,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{28}\text{H}_{36}\text{FN}_4\text{O}_5]^+$  requires 527.2670

The compound has not been reported previously.

### 9.67 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **203**



(*trans*)-2-Aminocyclohexan-1-ol **200** (1.04 g, 9.03 mmol, 1 eq.), TEA (1.65 mL, 1.20 g, 11.8 mmol, 1.3 eq.) and CH<sub>2</sub>Cl<sub>2</sub> (50 mL) were stirred at 0 °C. 4-Chlorobutyryl chloride **192** (1.22 mL, 1.54 g, 10.9 mmol, 1.2 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 30 min, then water (50 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with 10% *i*-PrOH/CHCl<sub>3</sub> (2×50 mL). The combined organic layers were dried with MgSO<sub>4</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, 0-100% EtOAc/Et<sub>2</sub>O). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **203** was obtained as white needles (1.51 g, 6.87 mmol, 76%).

**TLC**  $R_f$  = 0.19 (Et<sub>2</sub>O)

**mp**  $T$  / °C = 73-76 (*i*-PrOH, CHCl<sub>3</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3290 (N-H), 3250 (O-H), 2928 (C-H), 2857 (C-H), 1629 (amide C=O)

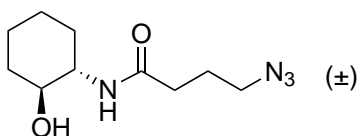
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.60 (t,  $J$  = 6.6 Hz, 2 H, CH<sub>2</sub>Cl), 3.51 - 3.60 (m, 1 H, CHNH), 3.28 - 3.39 (m, 1 H, CHOH), 2.37 (td,  $J$  = 7.4, 2.3 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.06 (quin,  $J$  = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.97 - 2.01 (m, 1 H, CHHCHOH), 1.85 - 1.93 (m, 1 H, CHHCHNH), 1.70 - 1.77 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.64 - 1.70 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.24 - 1.35 (m, 3 H, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH and CHHCHOH), 1.13 - 1.25 (m, 1 H, CHHCHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.0 (C(=O)), 74.1 (CHOH), 56.3 (CHNH), 45.3 (CH<sub>2</sub>Cl), 35.6 (CH<sub>2</sub>CHOH), 34.5 (C(=O)CH<sub>2</sub>), 32.7 (CH<sub>2</sub>CHNH), 30.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 242.0925, [M+Na]<sup>+</sup> found, [C<sub>10</sub>H<sub>18</sub>ClNNaO<sub>2</sub>]<sup>+</sup> requires 242.0924

The compound has not been reported previously.

### 9.68 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **204**



4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **203** (345 mg, 1.57 mmol, 1 eq.) and NaN<sub>3</sub> (180 mg, 2.77 mmol, 1.75 eq.) were stirred in DMF (12 mL) at 50 °C for 16 h. Water (50 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (50 mL) were added, and the organic layer was removed. The aqueous layer was extracted again with 10% *i*-PrOH/CHCl<sub>3</sub> (50 mL) and the combined organic fractions were dried with MgSO<sub>4</sub>. The solvent was evaporated under reduced pressure, and then by using a N<sub>2</sub> stream. **204** was obtained as large white prisms (347 mg, 1.53

mmol, 98%).

**TLC**  $R_f$  = 0.23 (EtOAc)

**mp**  $T$  / °C = 75-76 (*i*-PrOH, CHCl<sub>3</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3299 (N-H), 3208 (O-H), 2944 (C-H), 2928 (C-H), 2859 (C-H), 2089 (azide), 1624 (amide C=O)

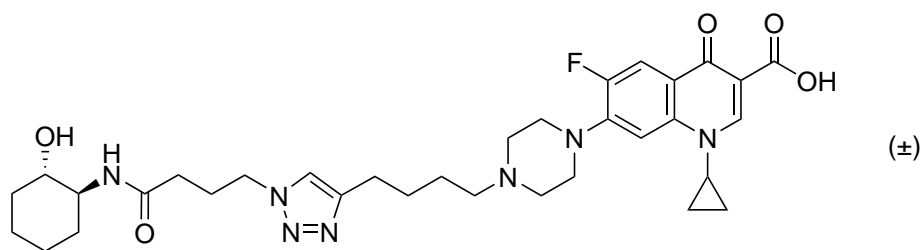
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 7.87 (d,  $J$  = 7.9 Hz, 1 H, NH), 5.27 (d,  $J$  = 4.3 Hz, 1 H, OH), 3.56 (td,  $J$  = 10.5, 4.4 Hz, 1 H, CHNH), 3.28 - 3.41 (m, 3 H, CHOH and CH<sub>2</sub>N<sub>3</sub>), 2.30 (td,  $J$  = 7.4, 2.7 Hz, 2 H, C(=O)CH<sub>2</sub>), 1.95 - 2.03 (m, 1 H, CHHCHOH), 1.87 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub> and CHHCHNH), 1.70 - 1.76 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.63 - 1.70 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.25 - 1.38 (m, 3 H, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH and CHHCHOH), 1.14 - 1.24 (m, 1 H, CHHCHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.1 (C(=O)), 74.0 (CHOH), 56.3 (CHNH), 52.0 (CH<sub>2</sub>N<sub>3</sub>), 35.5 (CH<sub>2</sub>CHOH), 34.3 (C(=O)CH<sub>2</sub>), 32.7 (CH<sub>2</sub>CHNH), 26.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 249.1331, [M+Na]<sup>+</sup> found, [C<sub>10</sub>H<sub>18</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 249.1327

The compound has not been reported previously.

### 9.69 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **205**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (40 mg, 97.2  $\mu$ mol, 1 eq.) and 4-azido-*N*-(((*trans*)-2-hydroxycyclohexyl)butanamide **204** (22.0 mg, 97.2  $\mu$ mol, 1 eq.) were dissolved in 10% water/*t*-BuOH (3 mL), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (97.2  $\mu$ l, 9.72  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (194  $\mu$ l, 19.4  $\mu$ mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at r.t. under argon for 16 h. Water (50 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (50 mL) were added, then the organic layer was separated, dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-70% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 50 mL) and 10% *i*-PrOH/CHCl<sub>3</sub> (50 mL). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **205** was obtained as a white amorphous solid (30.3 mg, 47.5  $\mu$ mol, 49%).

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3345 (N-H), 2928 (C-H), 2860 (C-H), 2815 (C-H), 1727 (carboxylic acid C=O), 1642 (amide C=O), 1626 (quinolone C=O), 1619 (triazole)

**$^1\text{H}$  NMR** (400 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 8.64 (s, 1 H, *ortho* to C(=O)OH), 7.86 (d,  $J$  = 13.9 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H,  $\text{CH}=\text{CCH}_2$ ), 7.64 (d,  $J$  = 8.1 Hz, 1 H,  $\text{NH}$ ), 7.54 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.54 (d,  $J$  = 4.7 Hz, 1 H,  $\text{OH}$ ), 4.30 (t,  $J$  = 6.8 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 3.77 - 3.86 (m, 1 H,  $\text{NCH(CH}_2)_2$ ), 3.33 - 3.40 (m, 1 H,  $\text{CHNH}$ ), 3.31 (br t,  $J$  = 4.8 Hz, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 3.14 - 3.24 (m, 1 H,  $\text{CHOH}$ ), 2.63 (t,  $J$  = 7.4 Hz, 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.56 (br t,  $J$  = 4.6 Hz, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 2.38 (t,  $J$  = 6.9 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.04 - 2.08 (m, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.96 - 2.04 (m, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.78 - 1.87 (m, 1 H,  $\text{CHHCHOH}$ ), 1.69 - 1.78 (m, 1 H,  $\text{CHHCHNH}$ ), 1.63 (quin,  $J$  = 7.5 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.54 - 1.60 (m, 2 H,  $\text{CHHCH}_2\text{OH}$ ), 1.51 (quin,  $J$  = 7.4 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.28 - 1.35 (m, 2 H,  $\text{NCH(CH}_2)_2$ ), 1.11 - 1.22 (m, 5 H,  $\text{NCH(CH}_2)_2$ ,  $\text{CHHCHOH}$ ,  $\text{CHHCH}_2\text{CHOH}$  and  $\text{CH}_2\text{CH}_2\text{CHNH}$ ), 1.04 - 1.13 (m, 1 H,  $\text{CHHCHNH}$ )

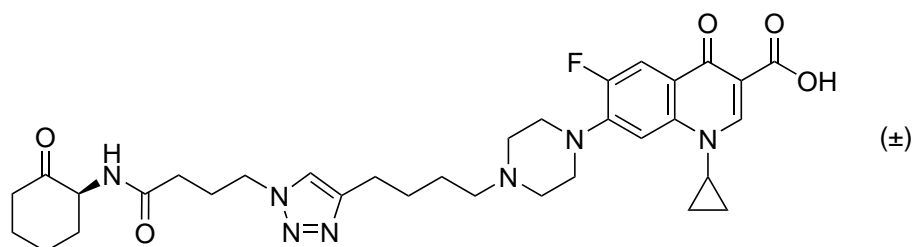
**$^{13}\text{C}$  NMR** (101 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 176.4 ( $\text{C(=O)CC(=O)OH}$ ), 170.9 ( $\text{CH}_2\text{C(=O)NH}$ ), 166.0 ( $\text{C(=O)OH}$ ), 153.1 (d,  $J$  = 252.1 Hz, *ipso* to F), 148.0 ( $\text{C}=\text{CC(=O)OH}$ ), 146.9 ( $\text{CH}=\text{CCH}_2$ ), 145.3 (d,  $J$  = 10.0 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 ( $\text{NCH}=\text{CCH}_2$ ), 118.5 (d,  $J$  = 8.3 Hz, *para* to piperazine), 110.9 (d,  $J$  = 23.2 Hz, *ortho* to C=O and *ortho* to F), 106.7 ( $\text{CC(=O)OH}$ ), 106.3 (d,  $J$  = 3.3 Hz, *meta* to C=O and *meta* to F), 71.4 ( $\text{CHOH}$ ), 57.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 54.2 ( $\text{CHNH}$ ), 52.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 48.8 ( $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{NCH}=\text{C}$ ), 35.9 ( $\text{NCH(CH}_2)_2$ ), 34.1 ( $\text{CH}_2\text{CHOH}$ ), 32.3 ( $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{NCH}=\text{C}$ ), 31.1 ( $\text{CH}_2\text{CHNH}$ ), 26.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 26.1 ( $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2\text{NCH}=\text{C}$ ), 25.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 25.0 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 24.2 ( $\text{CH}_2\text{CH}_2\text{CHNH}$ ), 23.8 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 7.6 ( $\text{NCH(CH}_2)_2$ )

**$^{19}\text{F}$  NMR** (376.45 MHz, DMSO  $d_6$ )  $\delta$  / ppm = -121.4 (ciprofloxacin F)

**HRMS** ( $\text{ESI}^+$ )  $m/z$  / Da = 638.3480,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{33}\text{H}_{45}\text{FN}_7\text{O}_5]^+$  requires 638.3466

The compound has not been reported previously.

## 9.70 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxocyclohexyl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **206**



1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **205** (15.0 mg, 23.6 mmol, 1 eq.) and Dess-Martin periodinane (35.0 mg, 82.5 mmol, 3.5 eq.) were stirred in  $\text{CH}_2\text{Cl}_2$  (3 mL) at r.t. for 4 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-70% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure, then  $\text{NaHCO}_3$  (aq., sat., 30 mL)

and 10% *i*-PrOH/CHCl<sub>3</sub> (30 mL) were added. The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **206** was obtained as a clear gum (11.7 mg, 18.4 μmol, 78%).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2941 (C-H), 2860 (C-H), 1720 (carboxylic acid C=O and ketone C=O), 1657 (amide C=O), 1626 (quinolone C=O), 1614 (triazole)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.65 (s, 1 H, *ortho* to C(=O)OH), 7.94 (d, *J* = 7.7 Hz, 1 H, NH), 7.88 (d, *J* = 13.4 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH<sub>2</sub>), 7.55 (d, *J* = 7.3 Hz, 1 H, *meta* to F), 4.40 (dddd, *J* = 12.8, 7.6, 6.1, 1.1 Hz, 1 H), 4.31 (t, *J* = 7.0 Hz, 1 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CHNH), 4.31 (t, *J* = 6.9 Hz, 1 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.74 - 3.84 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.31 (br. s, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (br t, *J* = 5.0, 5.0 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.45 - 2.52 (m, 1 H, CHHC(=O)), 2.38 (t, *J* = 7.1 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.25 (dtt, *J* = 13.4, 2.6, 1.6 Hz, 1 H, CHHC(=O)), 2.07 - 2.17 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N and CHHCNH), 1.96 - 2.05 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N and CHHCCH<sub>2</sub>C(=O)), 1.68 - 1.81 (m, 2 H, CHHCCH<sub>2</sub>CHNH), 1.64 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.40 - 1.56 (m, 5 H, CHHCCH<sub>2</sub>C(=O), CHHCNH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.27 - 1.34 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.13 - 1.20 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 207.4 (C(=O)CHNH), 176.3 (C(=O)CC(=O)OH), 170.8 (CH<sub>2</sub>C(=O)NH), 166.0 (C(=O)OH), 153.0 (d, *J* = 246.4 Hz, *ipso* to F), 147.9 (C=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.1 (d, *J* = 10.1 Hz, *ipso* to piperazine), 139.1 (*para* to F), 121.7 (NCH=CCH<sub>2</sub>), 118.7 (d, *J* = 6.9 Hz, *para* to piperazine), 110.9 (d, *J* = 23.0 Hz, *ortho* to C=O and *ortho* to F), 106.3 (CC(=O)OH, and *meta* to C=O and *meta* to F), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 57.0 (CHNH), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 40.5 (CH<sub>2</sub>C(=O)), 35.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.7 (CH<sub>2</sub>CHNH), 31.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 27.1 (CH<sub>2</sub>CH<sub>2</sub>C(=O)), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 23.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.7 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 636.3303, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 636.3310

The compound has not been reported previously.

## 9.71 Biological testing

Compounds were tested against *P. aeruginosa* PAO1<sup>180</sup> and YM64.<sup>181</sup> C<sub>4</sub>-HSL **19**, HHQ **21**, PQS **22**, ciprofloxacin **24**, trimethoprim **25** and DMSO were included as controls, along with LB to check for contamination of the plates.

The first set of autoinducer-antibiotic conjugates (see 7.5) were tested at 2, 1, 0.5, 0.25, 0.125 and 0.0625 μM. Breathe-Easy<sup>®</sup> sealing membranes from Diversified Biotech were used and the plates were placed without lids in a open box containing tissue paper wetted with distilled water in order to control evaporation. OD readings at 595 nm were taken at 5 and 24 h, and biofilm quantification was carried out soon after the 24 h OD reading. Crystal violet-stained plates were also read at 595 nm. Only a 5 h OD reading in YM64 was obtained for the cleavable HSL-Cip conjugates.

The HSL analogue-Cip(Me) conjugates (see 8.7) were tested at 25, 2, 1, 0.5, 0.25 and 0.125 μM in triplicate. AeraSeal<sup>TM</sup> films from Excel Scientific were used. A plate lid was used, but the humidified box was not. OD readings at 600 nm were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 h. Biofilm inhibition testing was carried



out on plates grown for 24 and 48 h. Biofilm dispersal testing was carried out by growing plates for 24 h, followed by addition of the compounds, incubation for a further 24 h and quantification of the biofilms. Crystal violet-stained plates were read at 550 nm.

#### **9.71.1 Antibiotic susceptibility**

Antibiotic susceptibility was determined using spectrophotometry measurements. Colonies of the desired strains were grown at 37 °C overnight on LB agar. The colonies were used to inoculate LB (10 mL) and these cultures were grown at 37 °C overnight. The cultures were diluted 1/100 with LB, and 99  $\mu$ l diluted culture per well was added to Nunclon® flat-bottomed clear 96-well plates. 1  $\mu$ l of compound solution in DMSO was then added from master plates and the plates were covered with adhesive aeration filters. The plates were shaken at 37 °C and 100 rpm and OD was recorded periodically using a Biochrom EZ Read 400 microplate reader.

#### **9.71.2 Quantification of biofilms**

Biofilms were quantified using a method described previously.<sup>126, 242</sup> After the bacteria had grown for the desired amount of time, the culture was aspirated out of the wells using a pipette tip attached to a vacuum pump, making sure not to touch the sides of the wells. Water (120  $\mu$ l) was then added and aspirated out again. This process was repeated twice more to thoroughly wash out planktonic cells. Crystal violet (120  $\mu$ l, 0.1% *m/v*) was added and left for 15 min, then aspirated out. The wells were washed again with water ( $3 \times 120 \mu$ l). Acetic acid (120  $\mu$ l, 30% *v/v* aq.) was added and left for 15 min then the plate was vortexed and read using a Biochrom EZ Read 400 microplate reader at 595 nm.

#### **9.71.3 Biofilm inhibition**

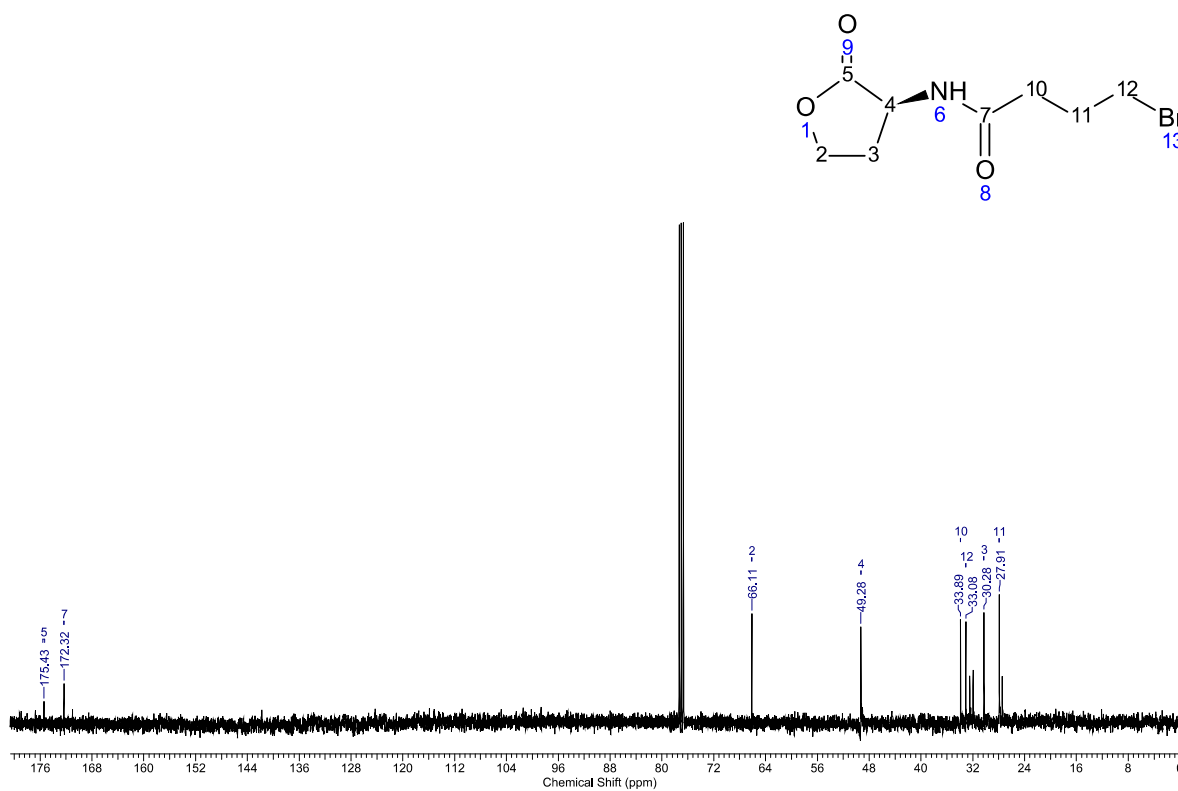
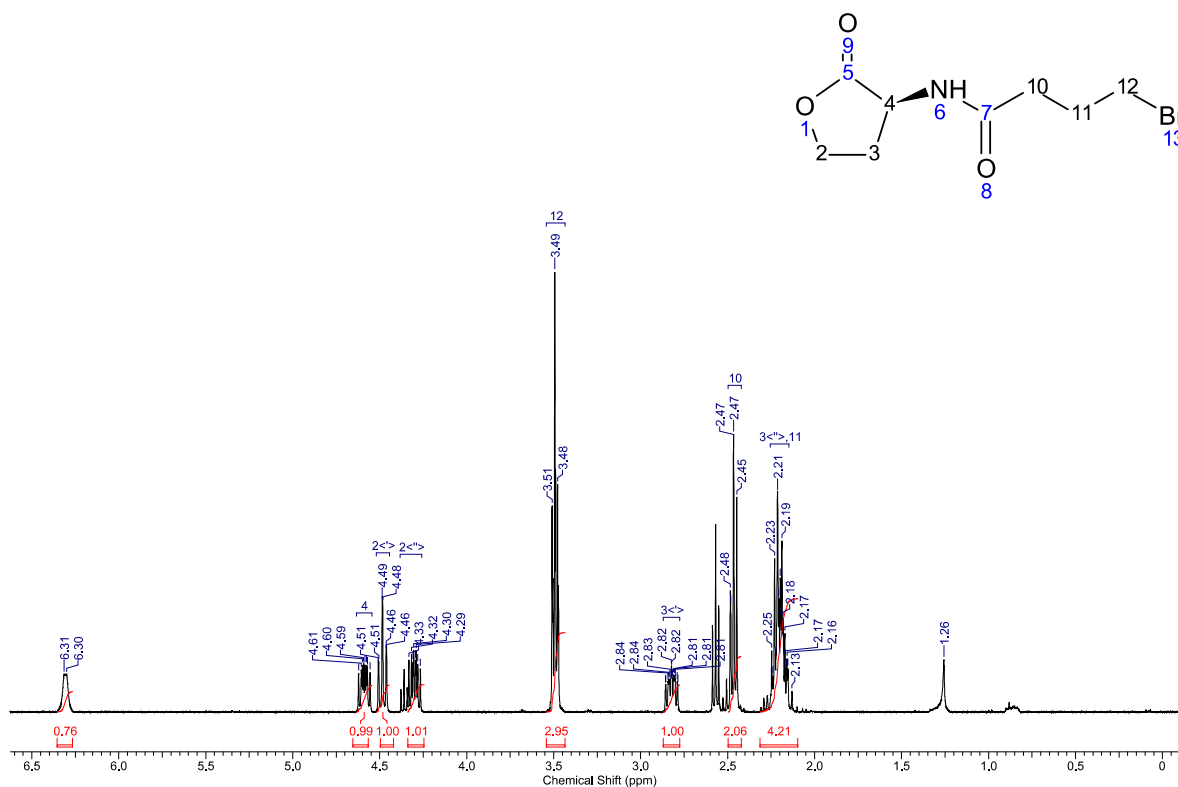
The plates were prepared as in 9.71.1. The plates were shaken at 37 °C and 100 rpm for 24 h followed by quantification of biofilm growth as shown in 9.71.2.

#### **9.71.4 Biofilm dispersal**

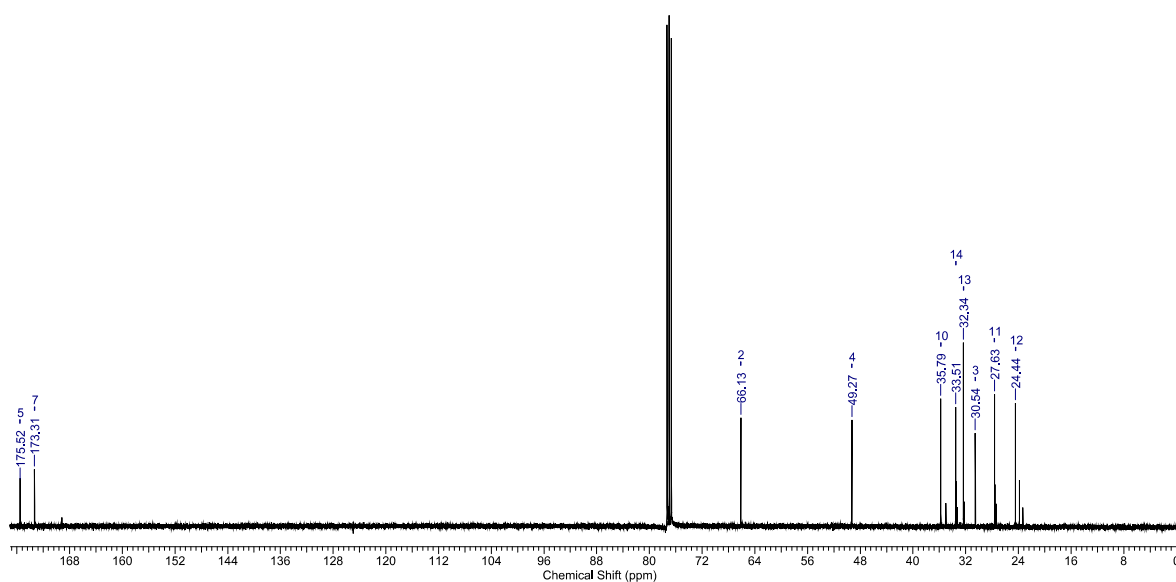
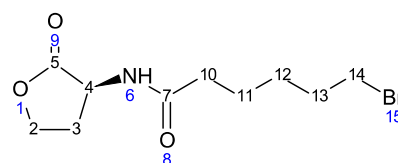
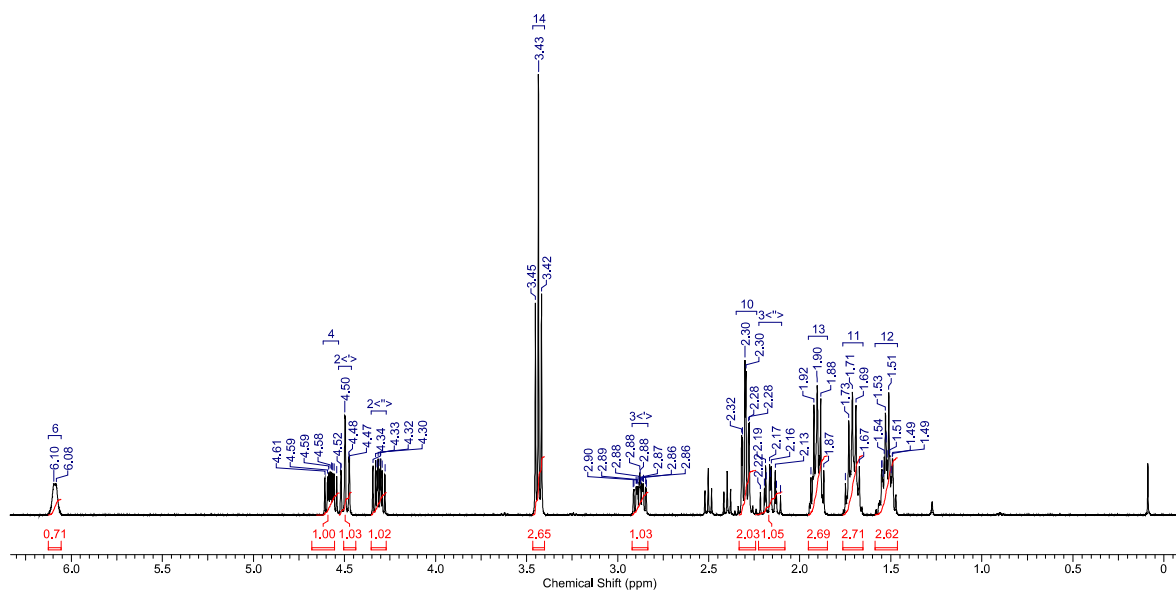
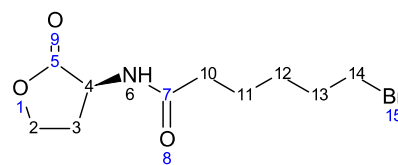
The plates were prepared as in 9.71.1, initially without the addition of compound solutions. The box of plates was shaken at 37 °C and 100 rpm for 24 h. 1  $\mu$ l of compound solution in DMSO was then added to each well from master plates and the plates were shaken as above for a further 24 h followed by measurement of OD and quantification of biofilm growth as shown in 9.71.2.

## 10 NMR spectra

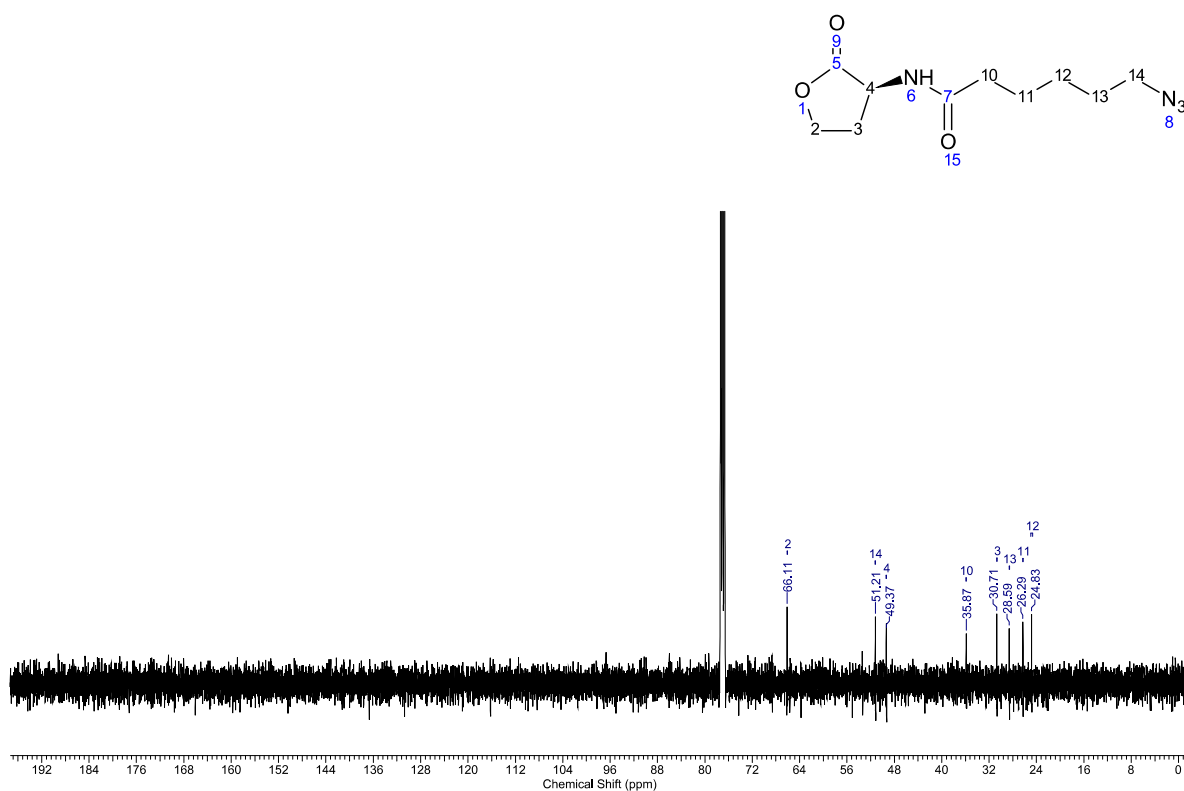
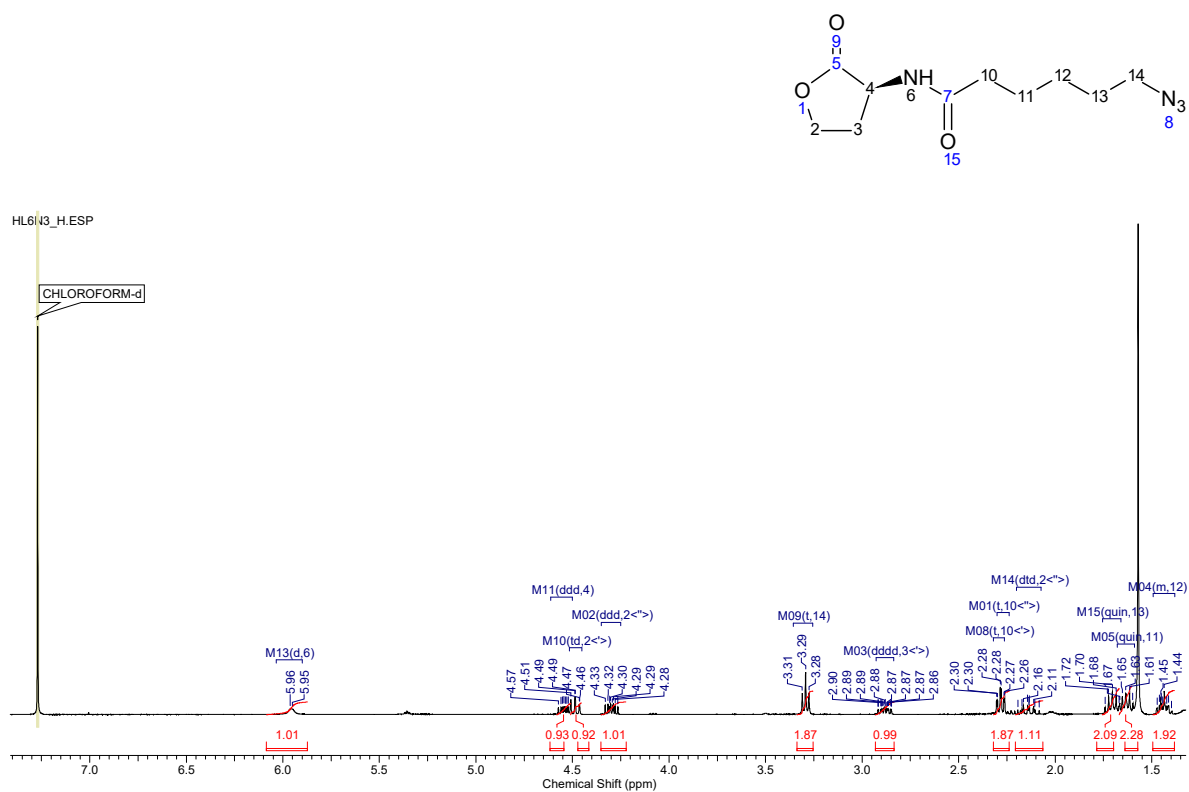
### 10.1 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 57



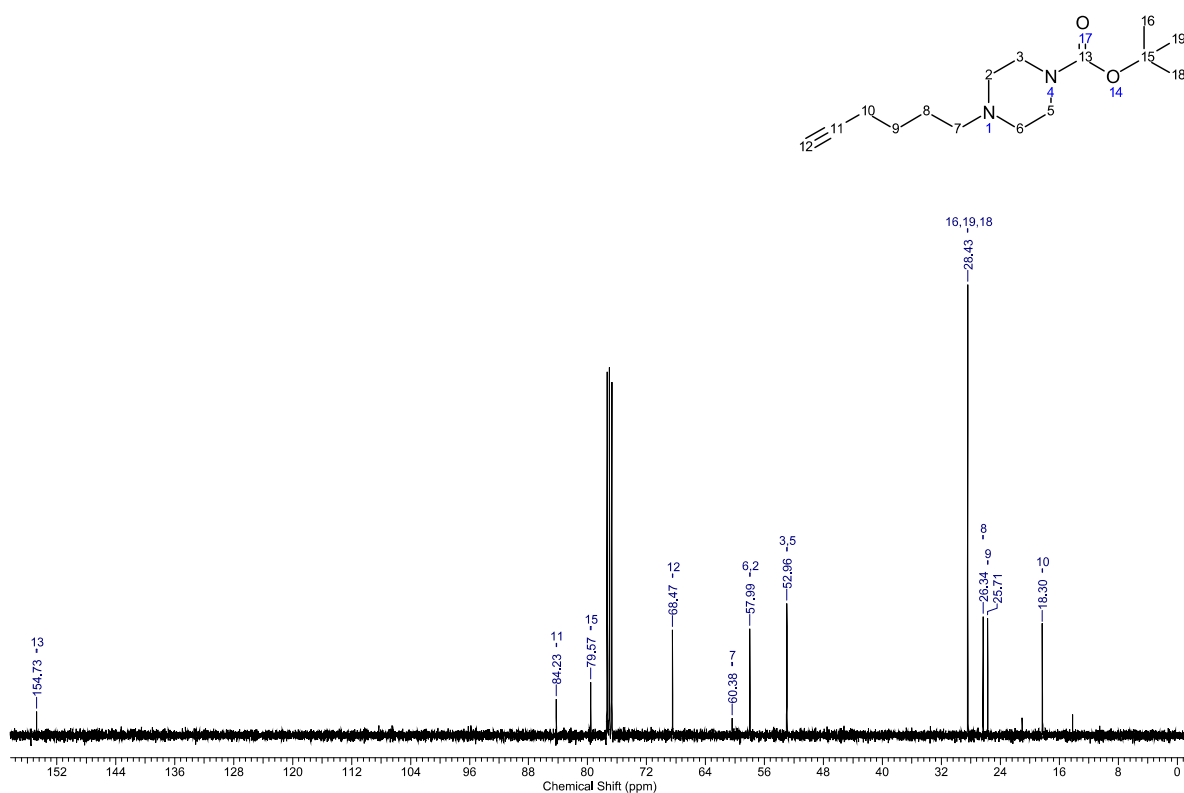
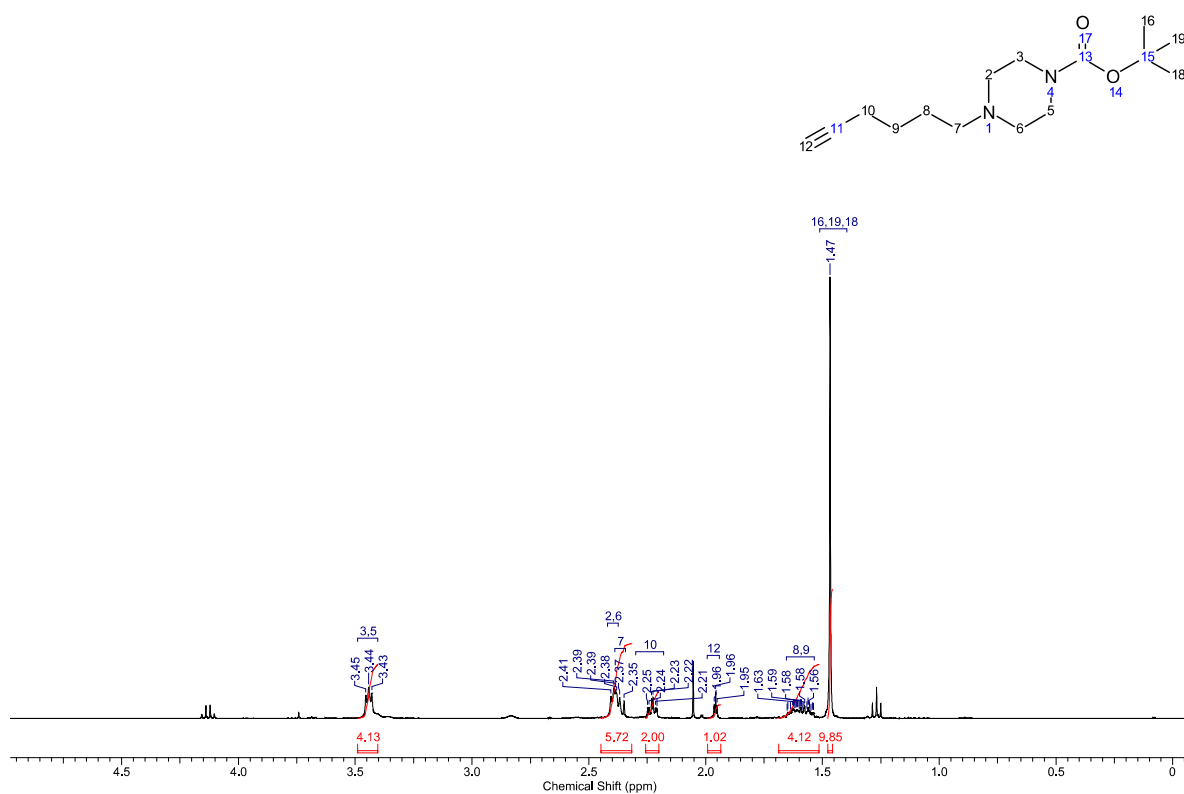
## 10.2 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 60



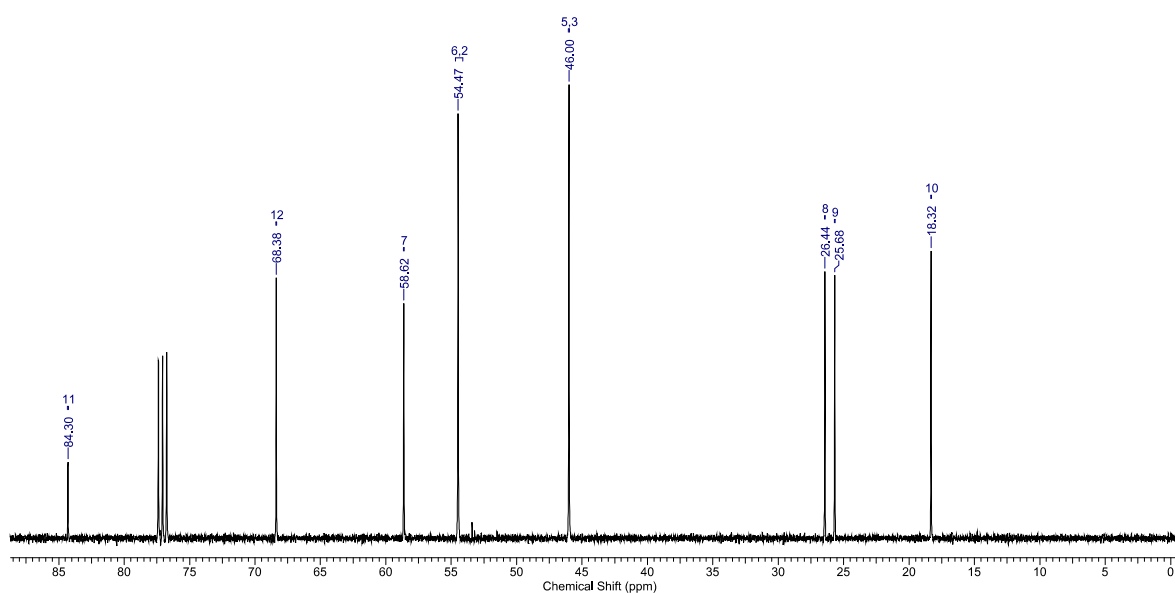
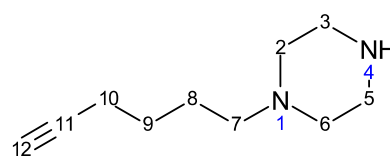
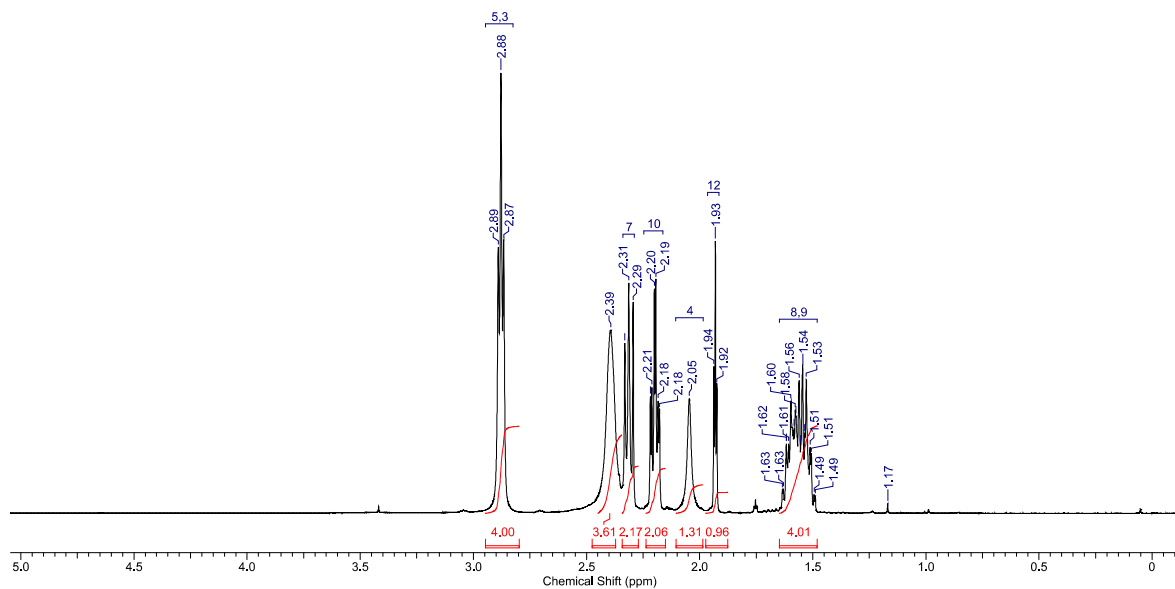
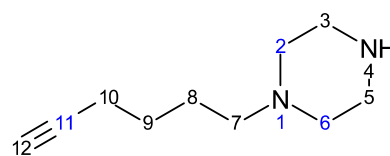
### 10.3 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 61



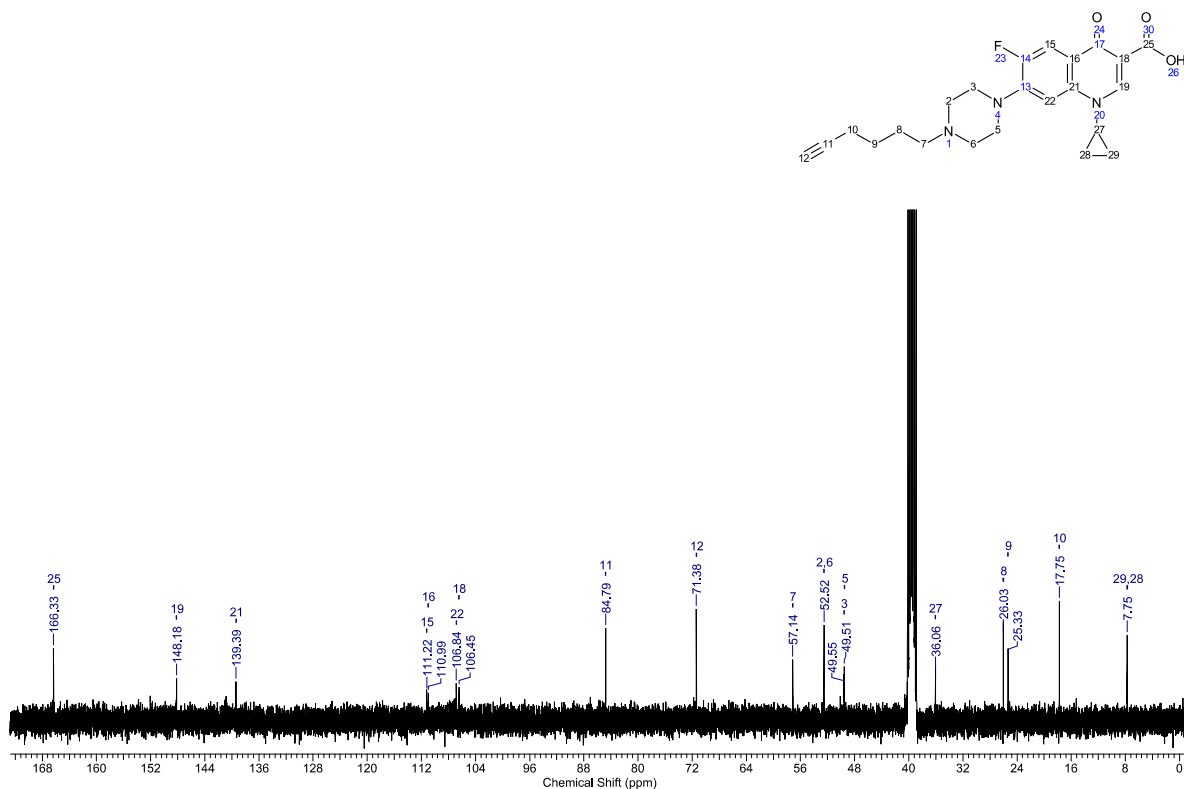
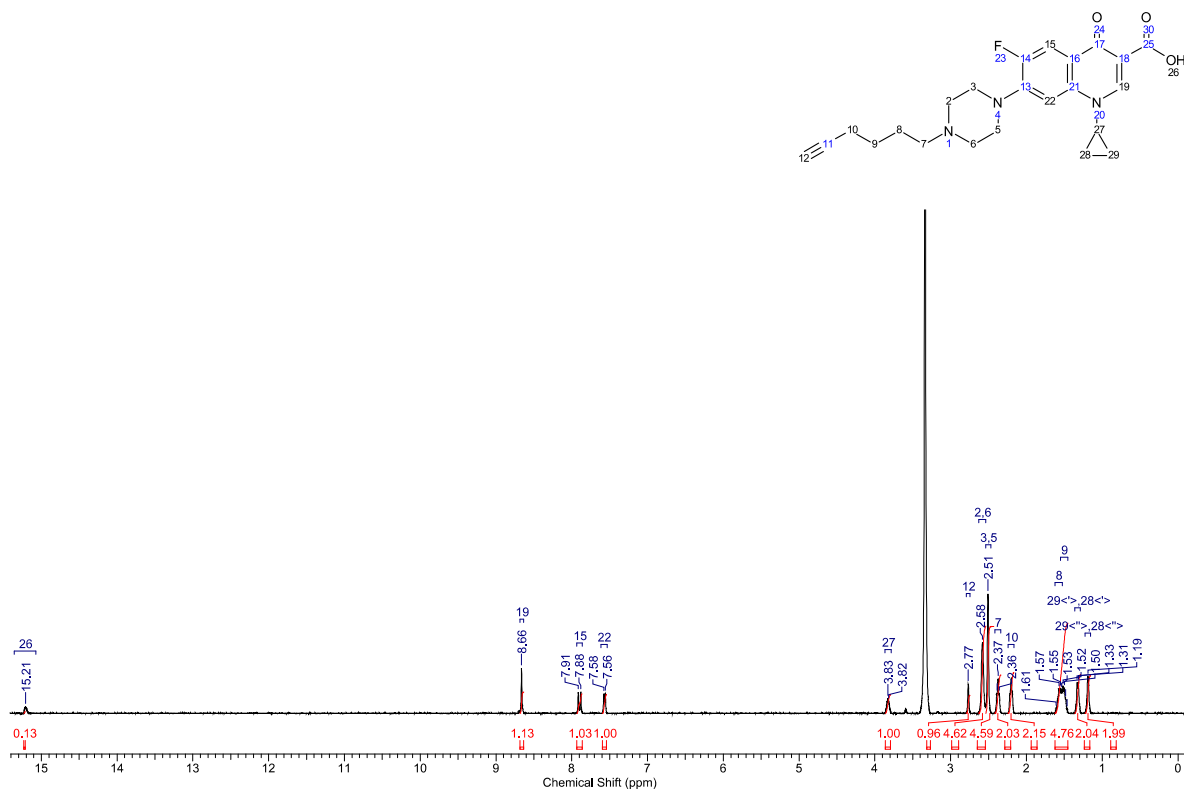
## 10.4 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate **65**



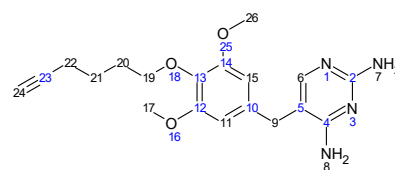
## 10.5 1-(Hex-5-yn-1-yl)piperazine 66



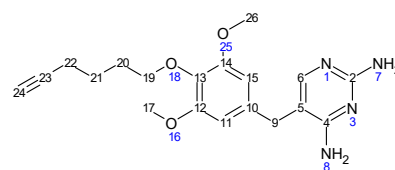
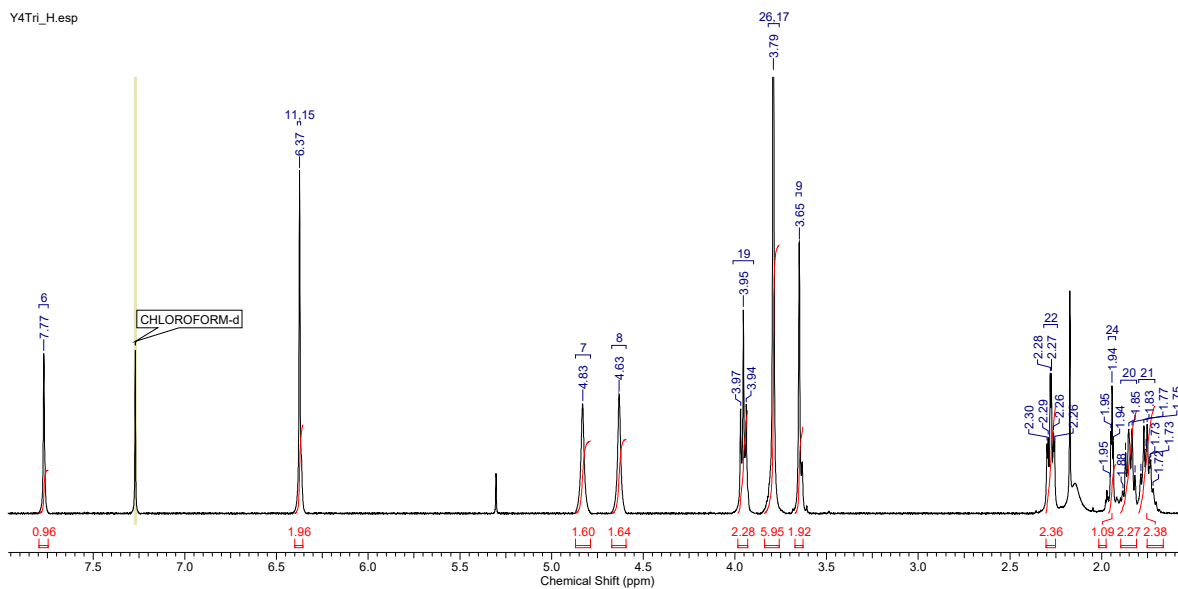
10.6 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68**



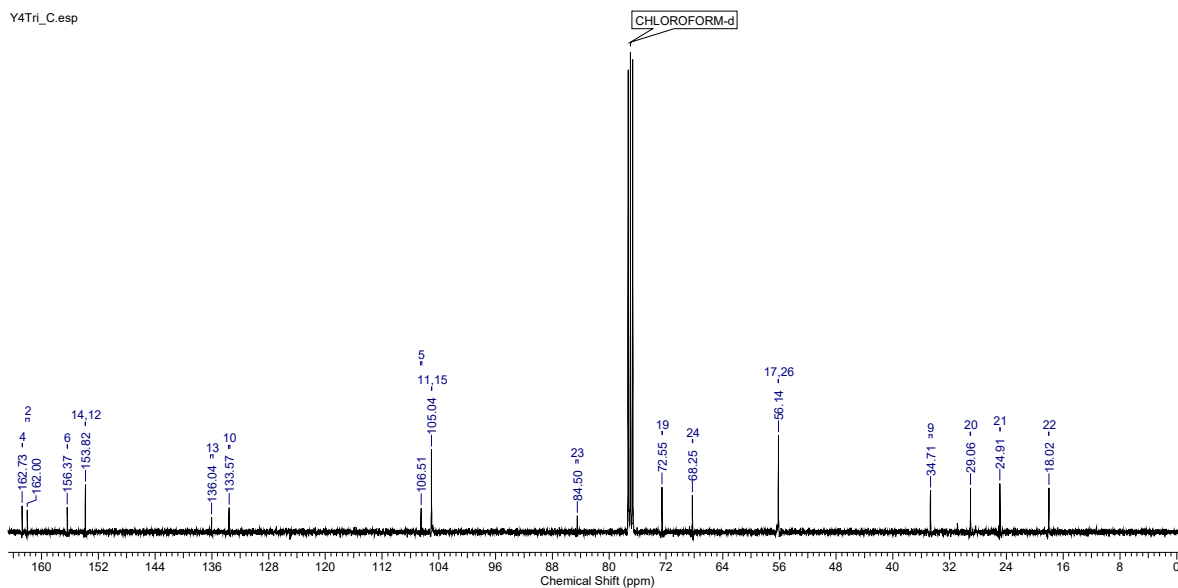
## 10.7 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71



Y4Tri\_H.esp

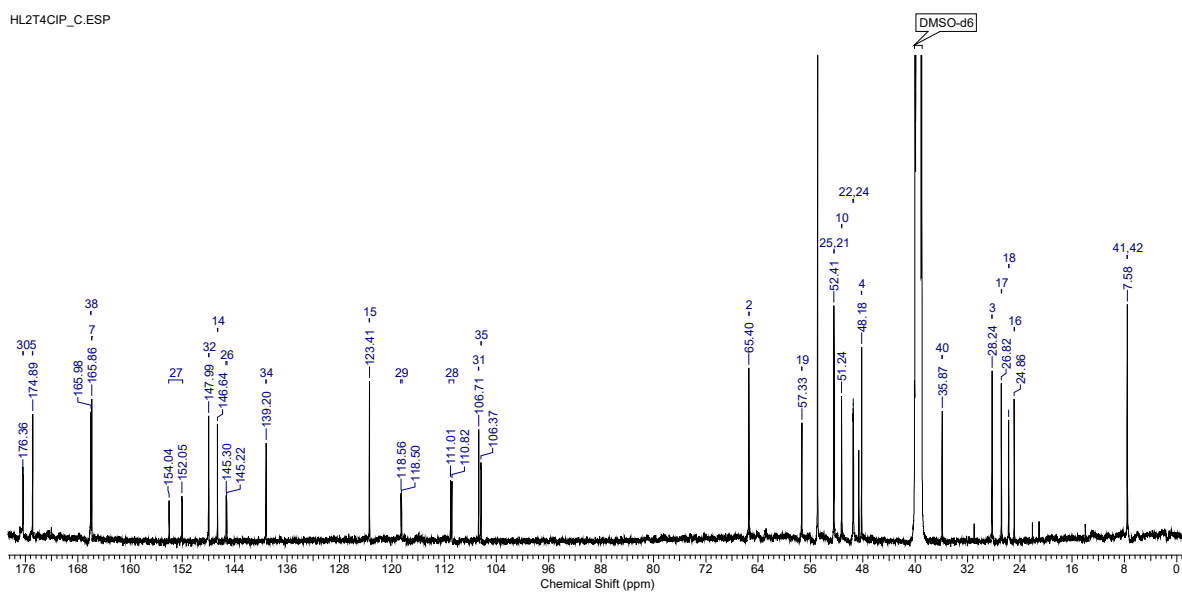
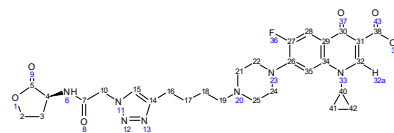
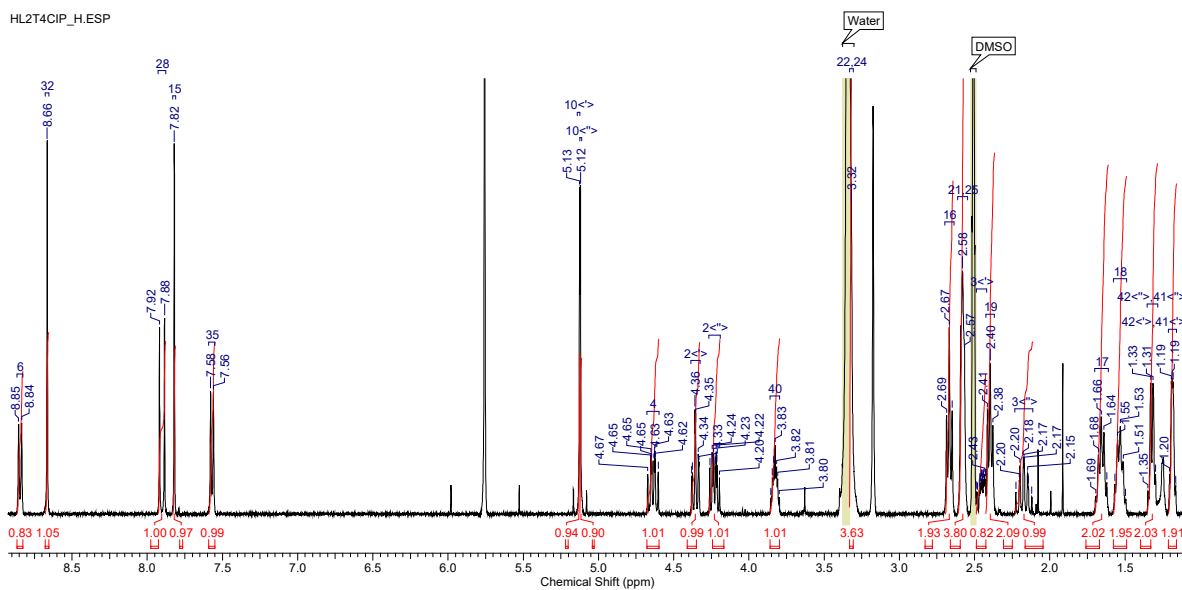
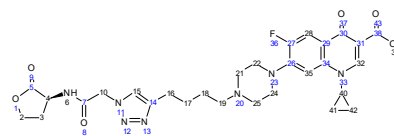


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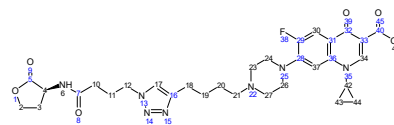




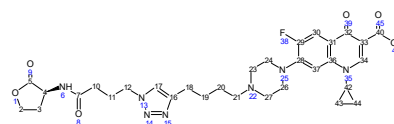
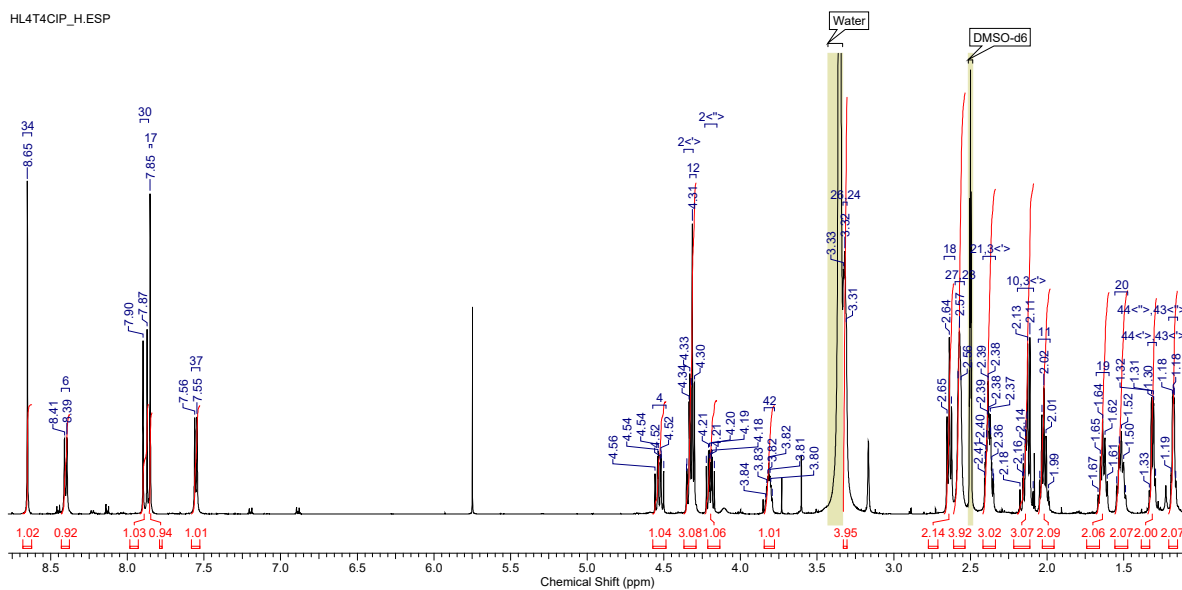
10.8 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **72**



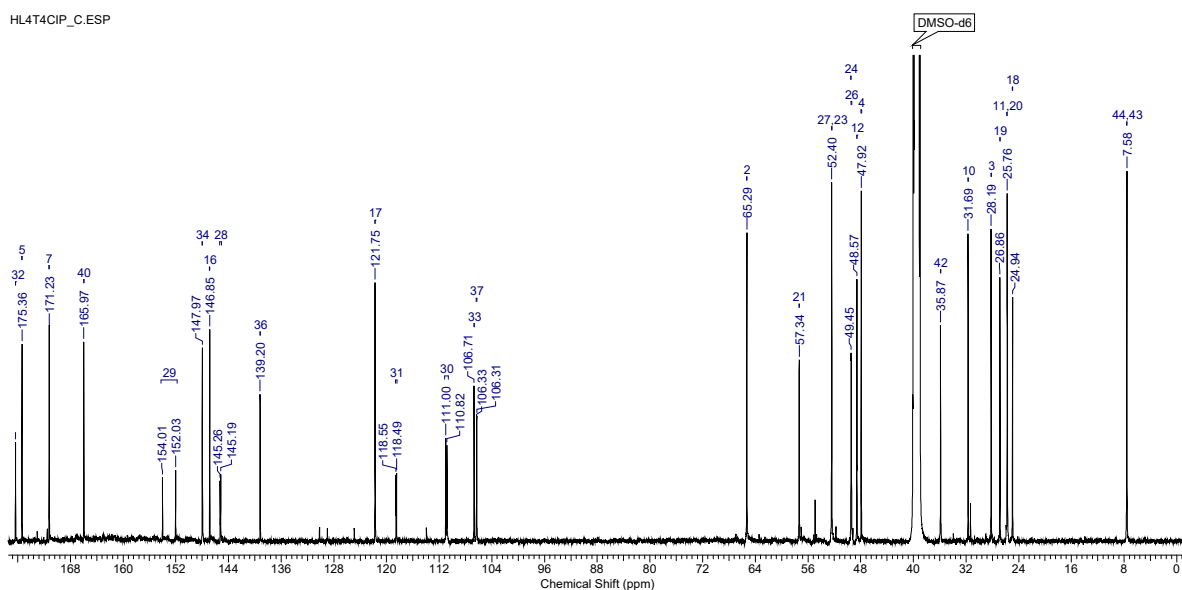
10.9 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77



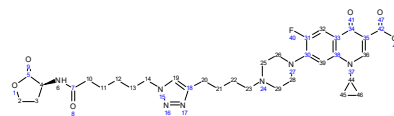
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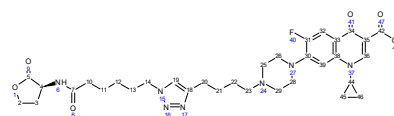
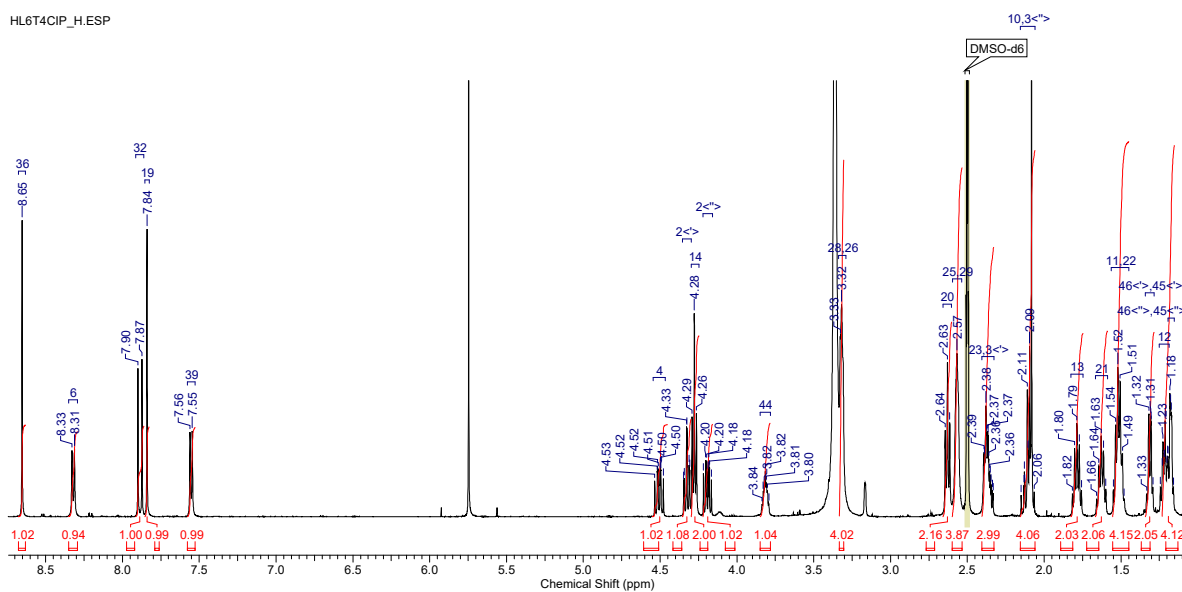
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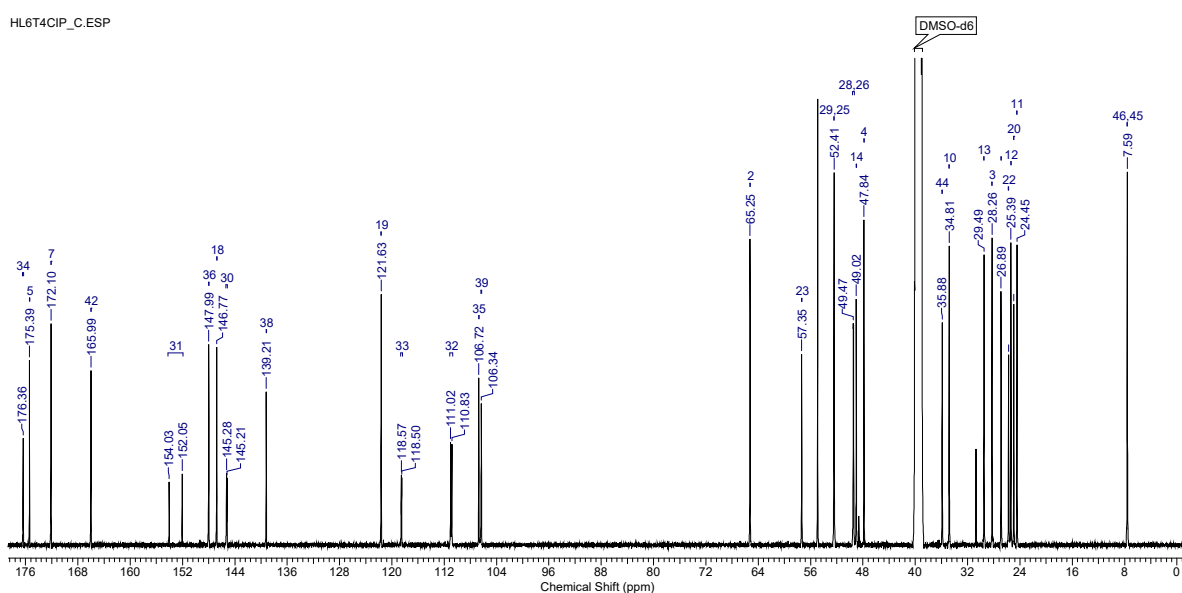
10.10 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **78**



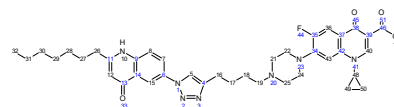
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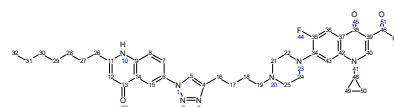
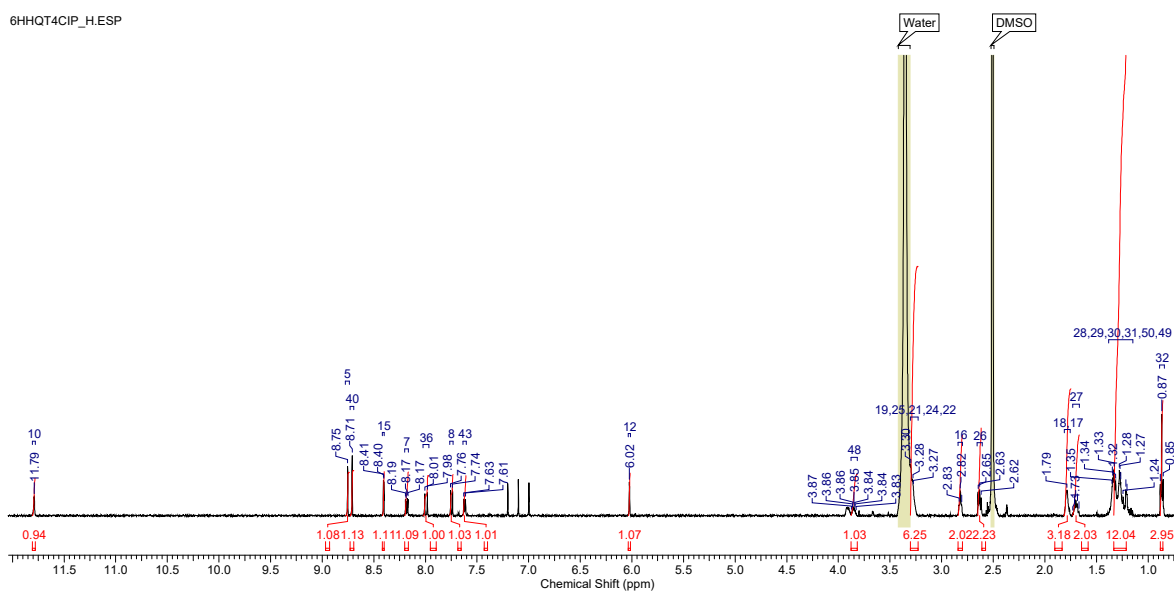
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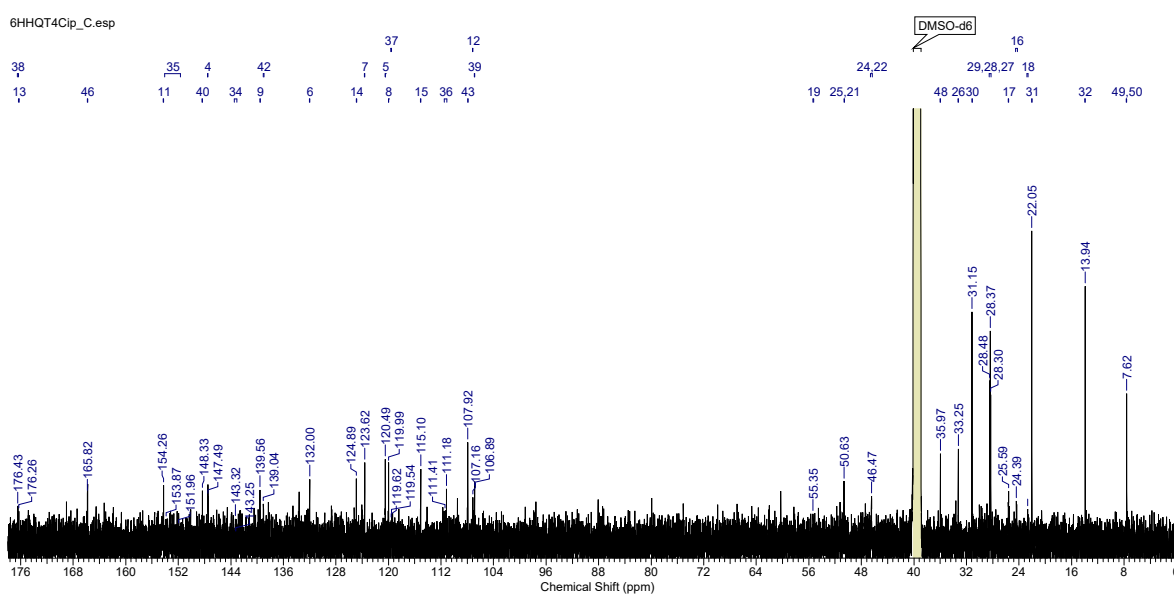
10.11 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 80



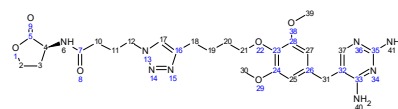
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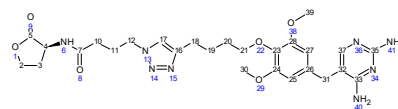
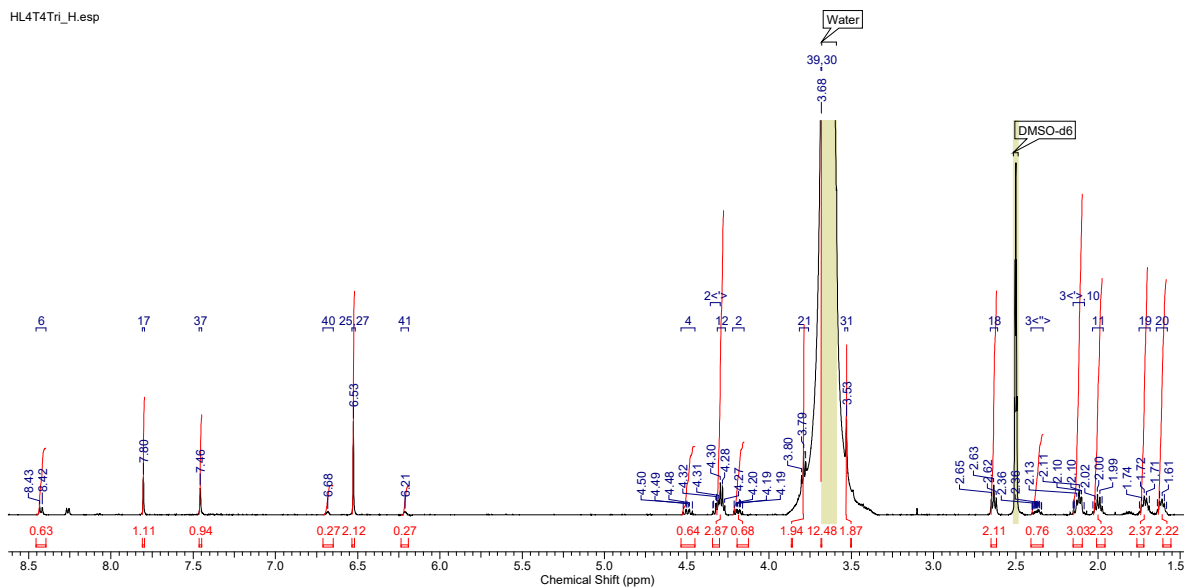
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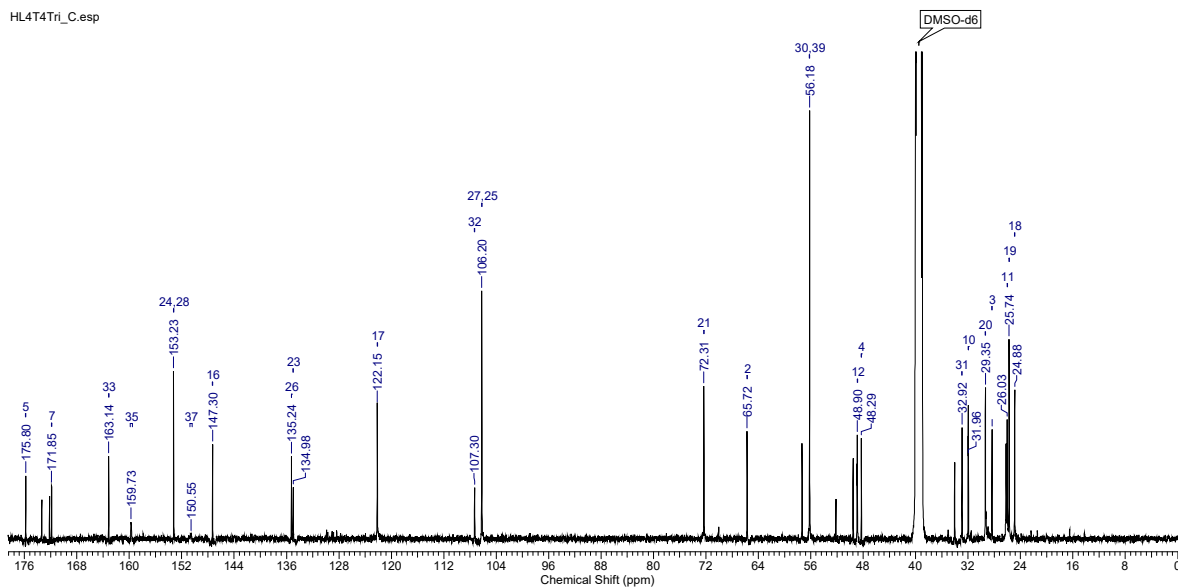
10.12 (*S*)-4-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 84



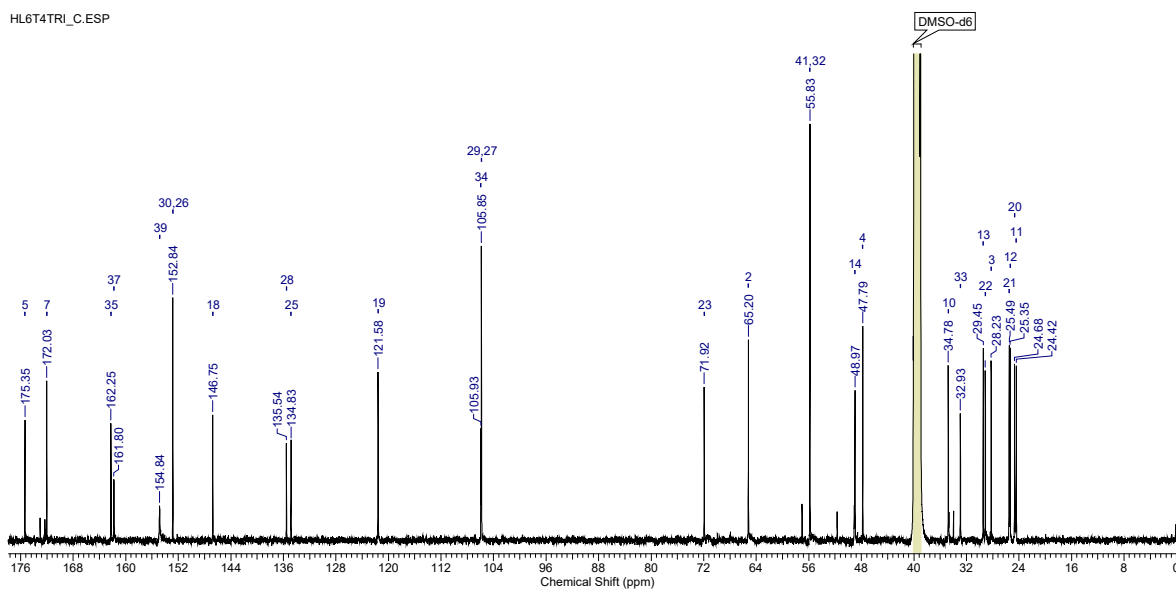
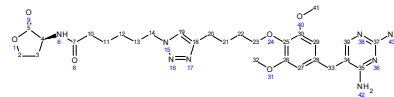
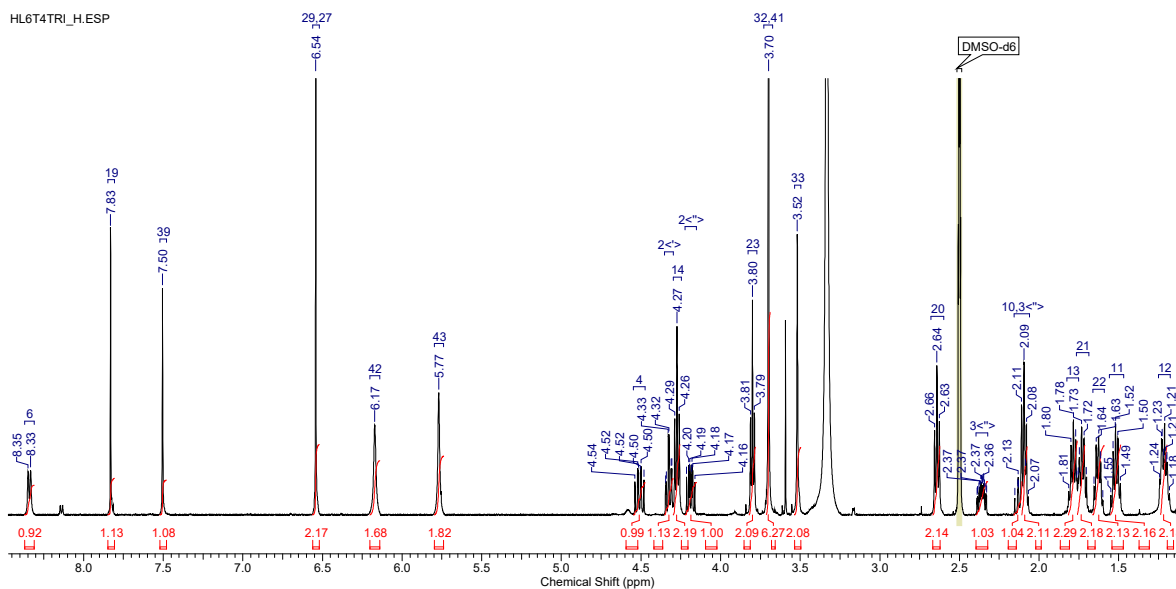
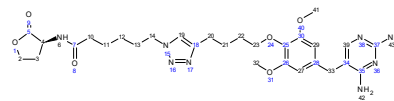
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HL4T4Tri\_C.esp

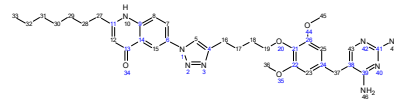


10.13 (*S*)-6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 85

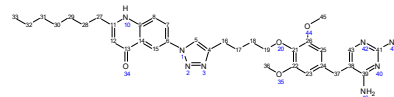
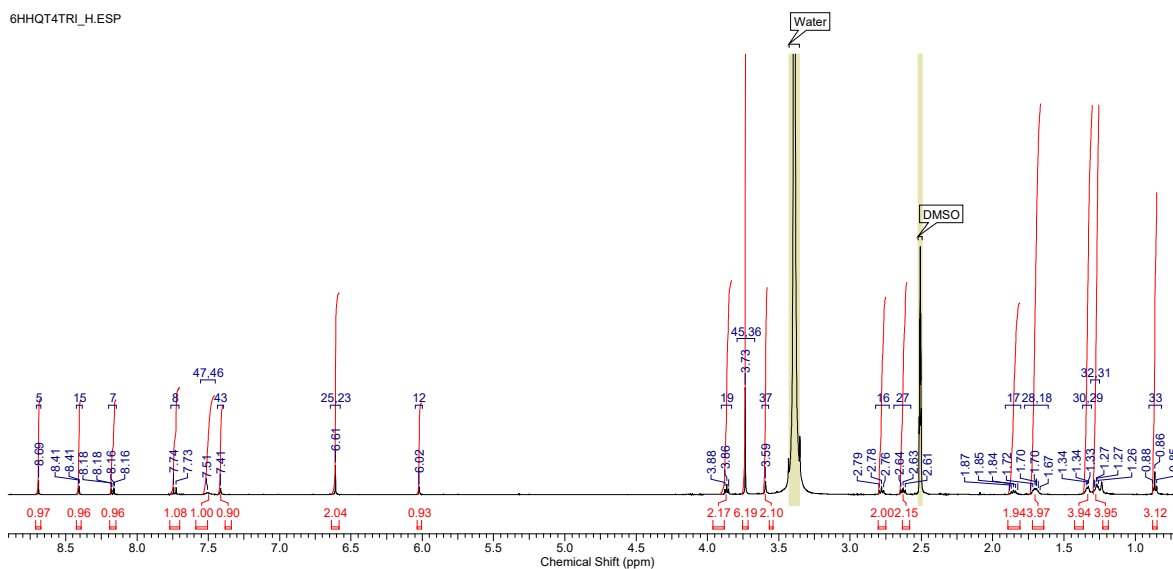


10.14 6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(1*H*)-one 87

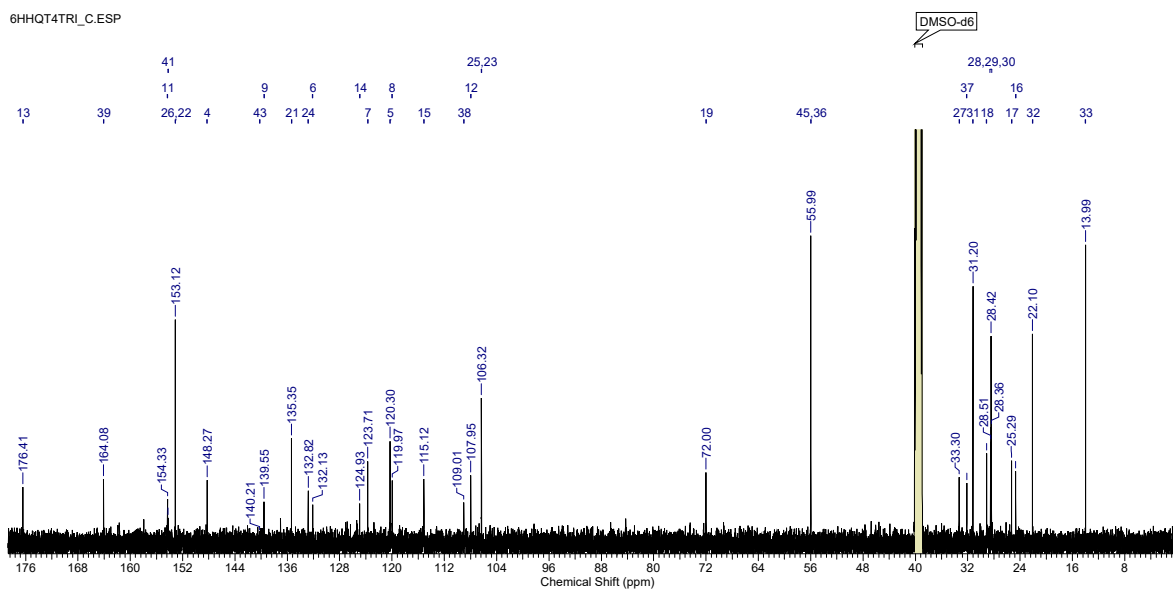
User Notes Some guesses



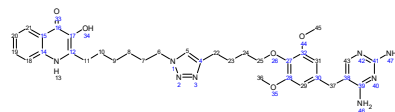
6HHQT4TRI\_H.ESP



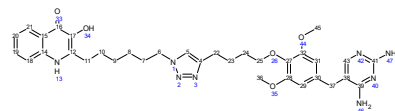
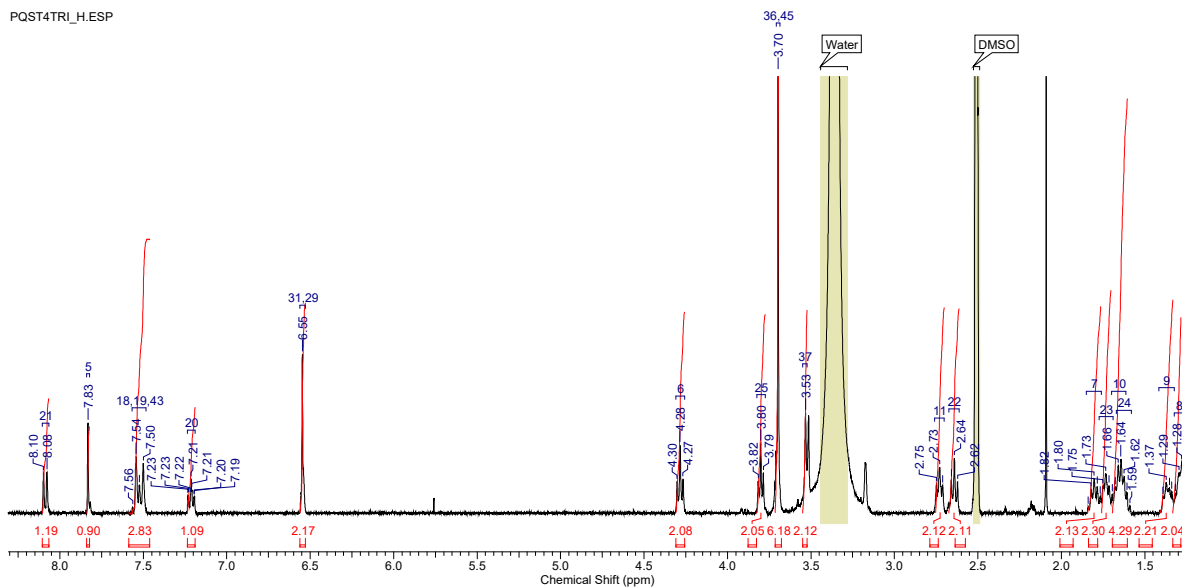
6HHQT4TRI\_C.ESP



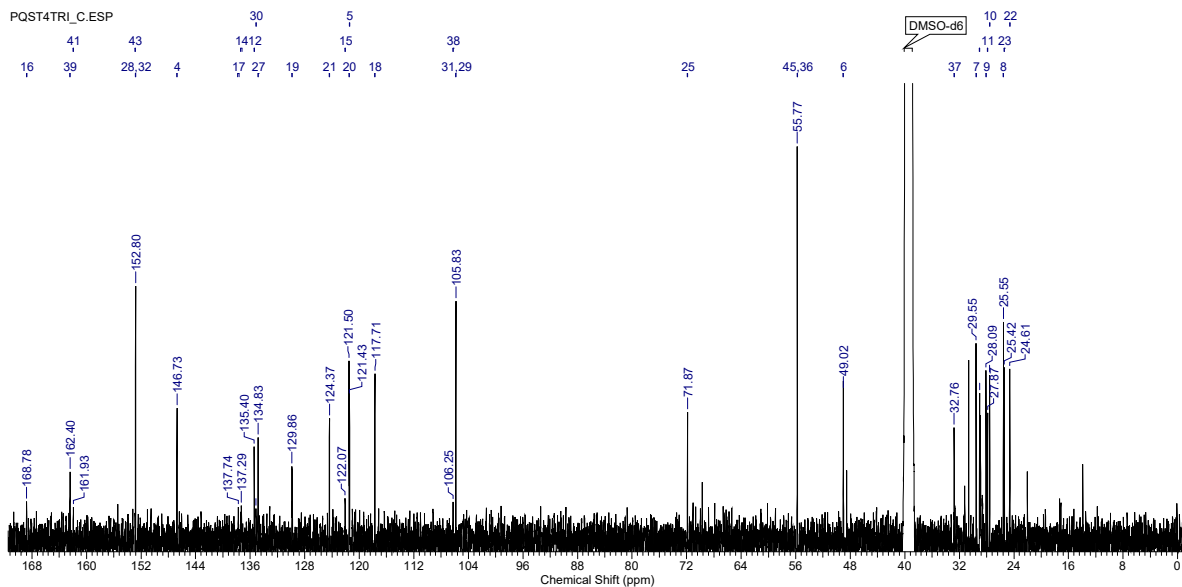
10.15 2-(6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(1*H*)-one 89



PQST4TRI\_H.ESP

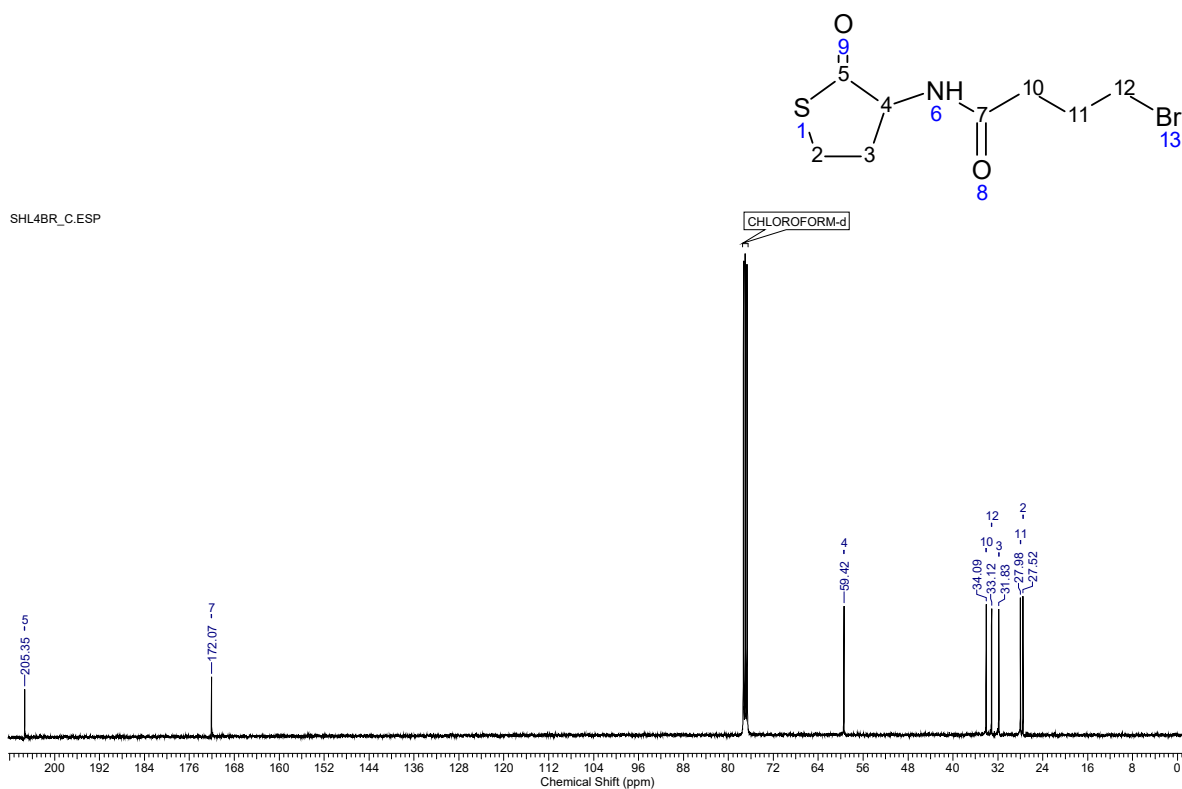
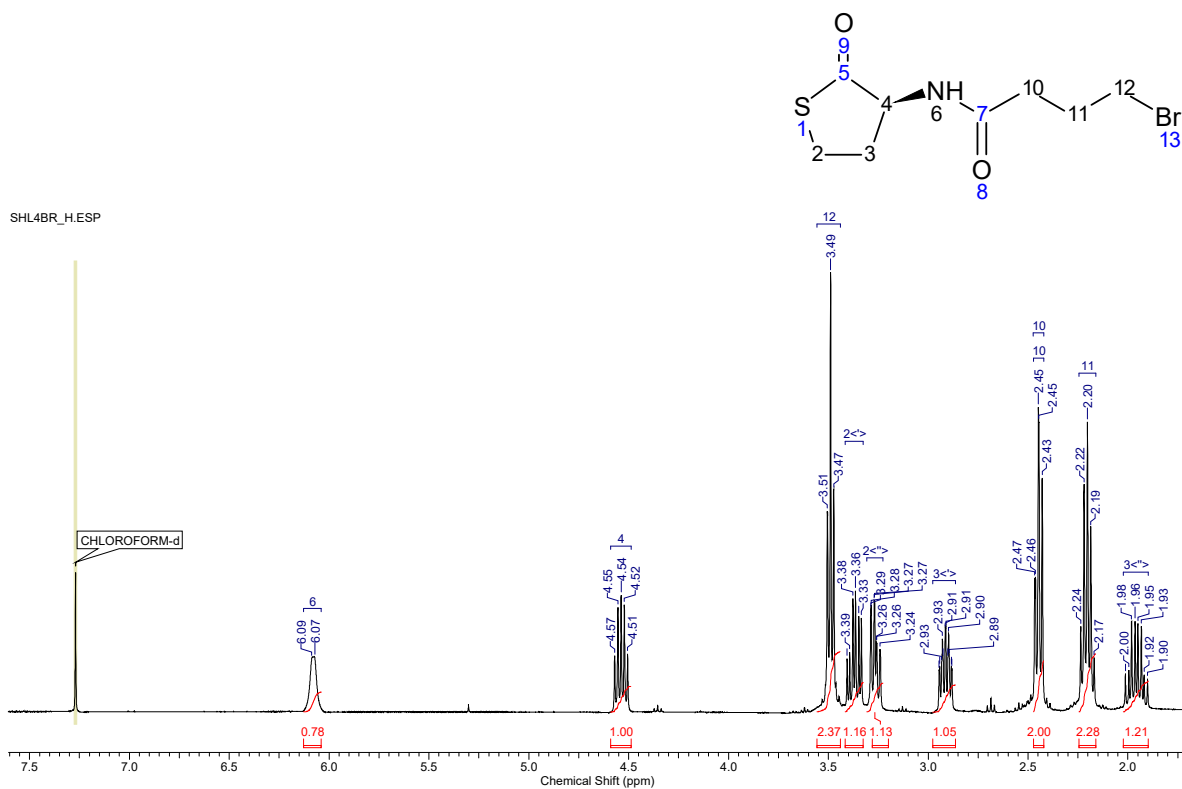


PQST4TRI\_C.ESP

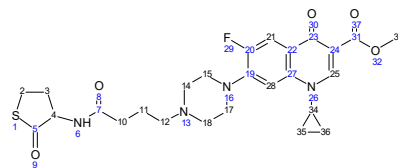




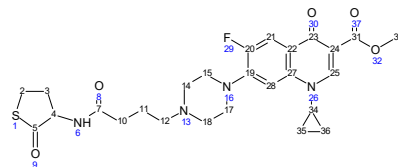
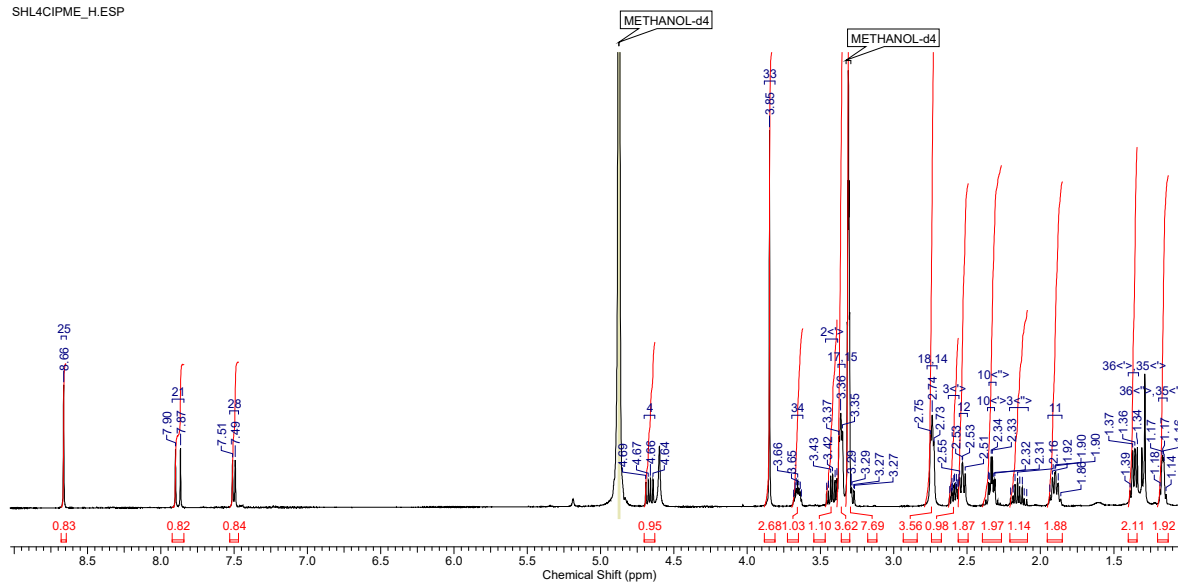
# 10.16 4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 153



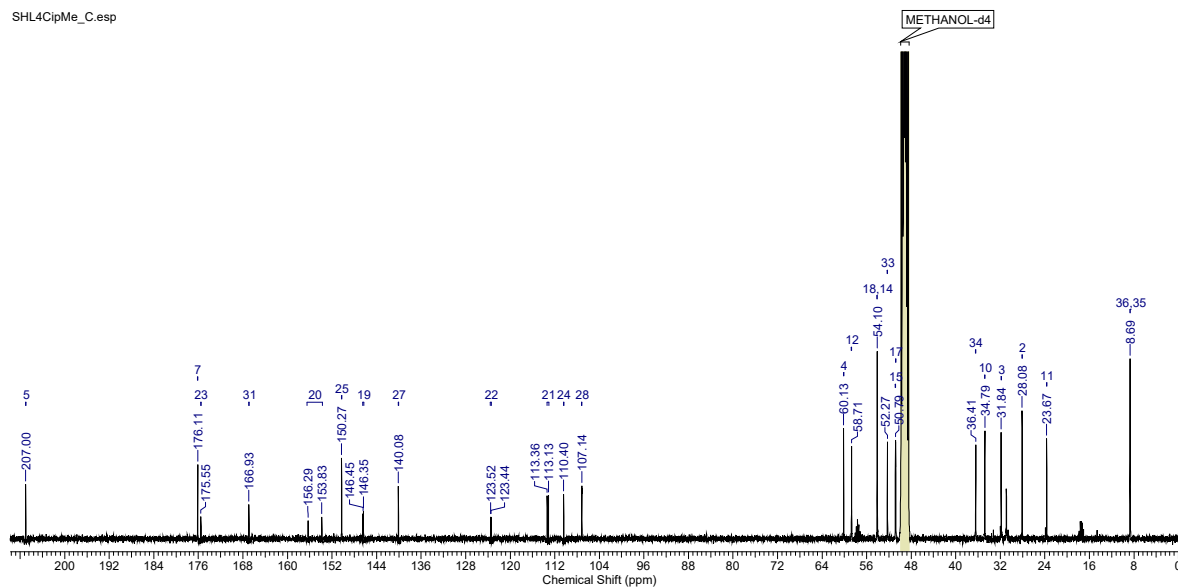
## 3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 154



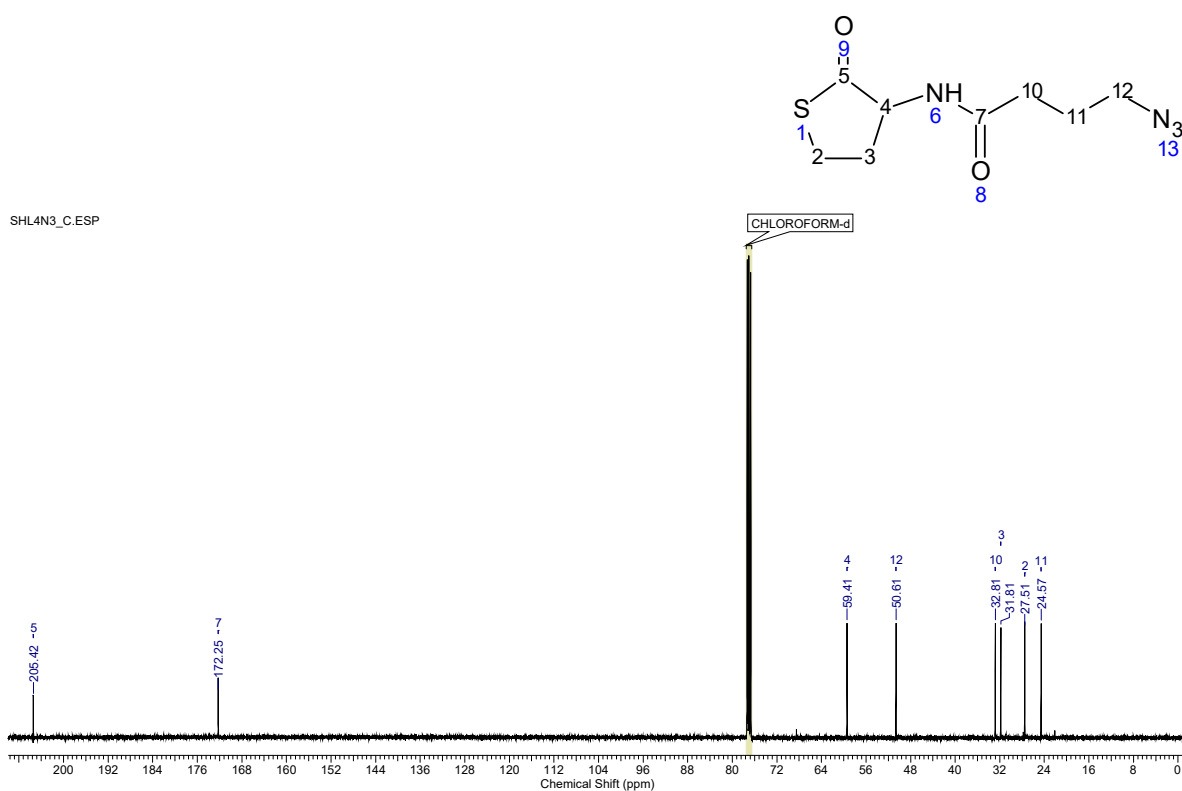
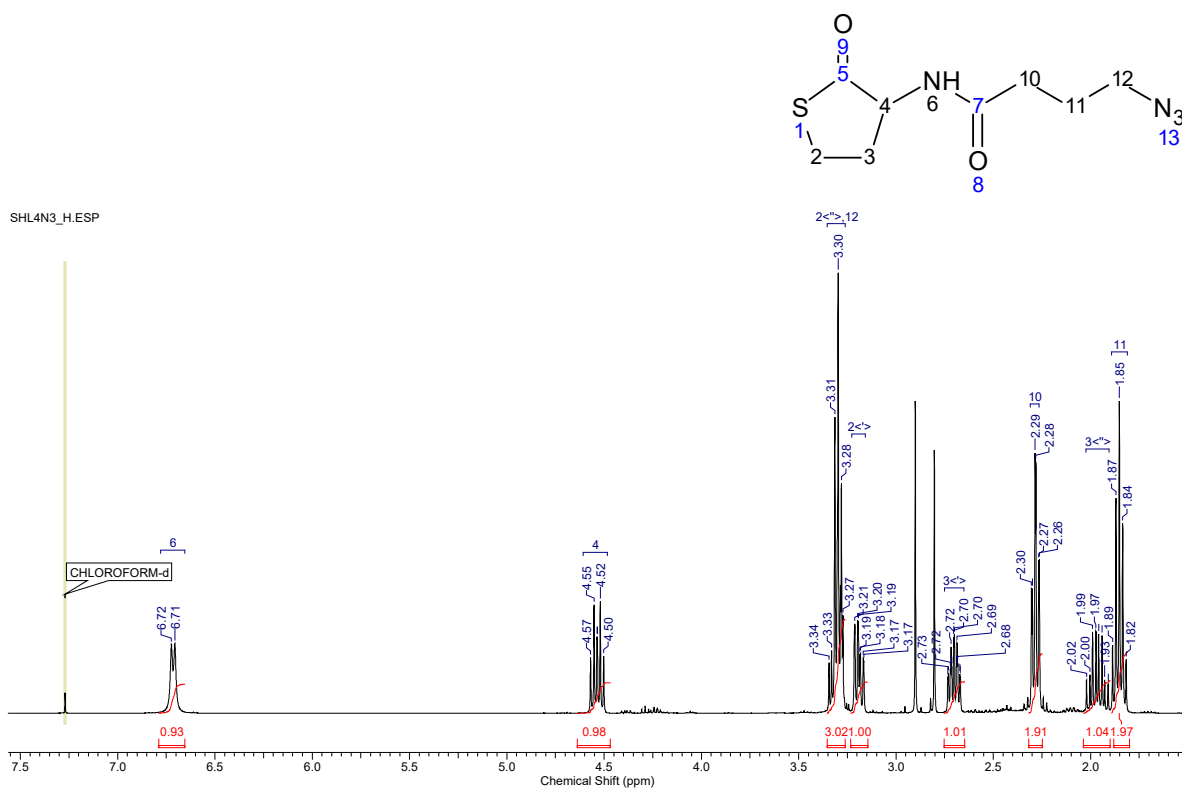
SHL4CIPME\_H.ESP



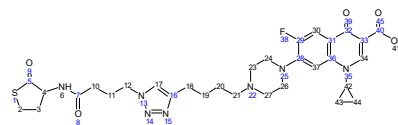
SHL4CipMe\_C.esp



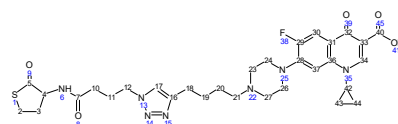
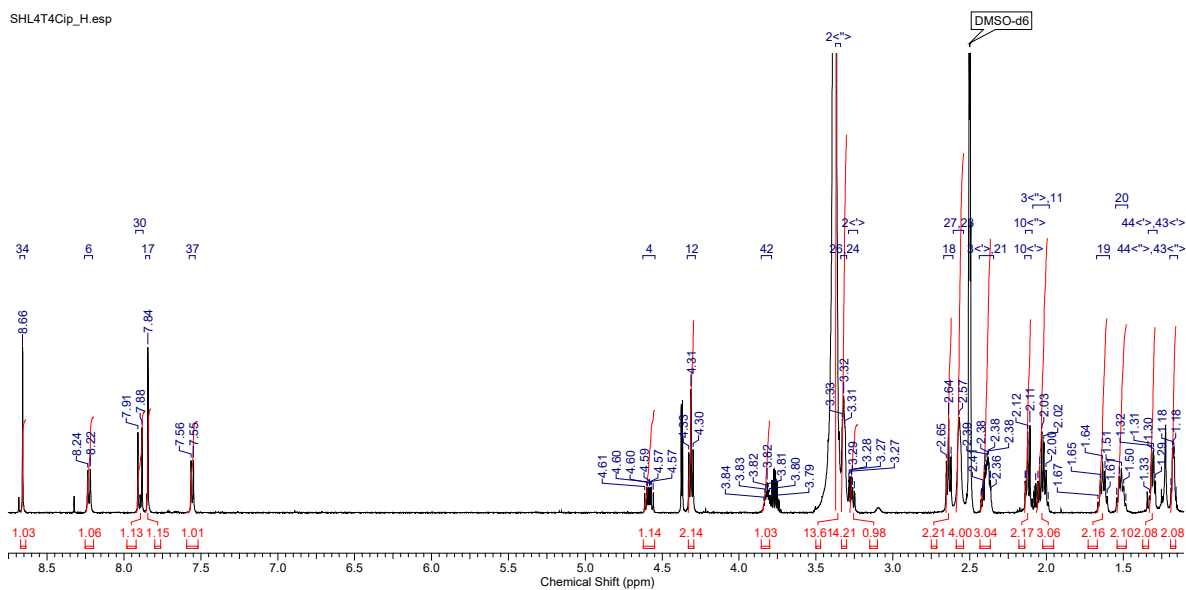
# 10.18 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 155



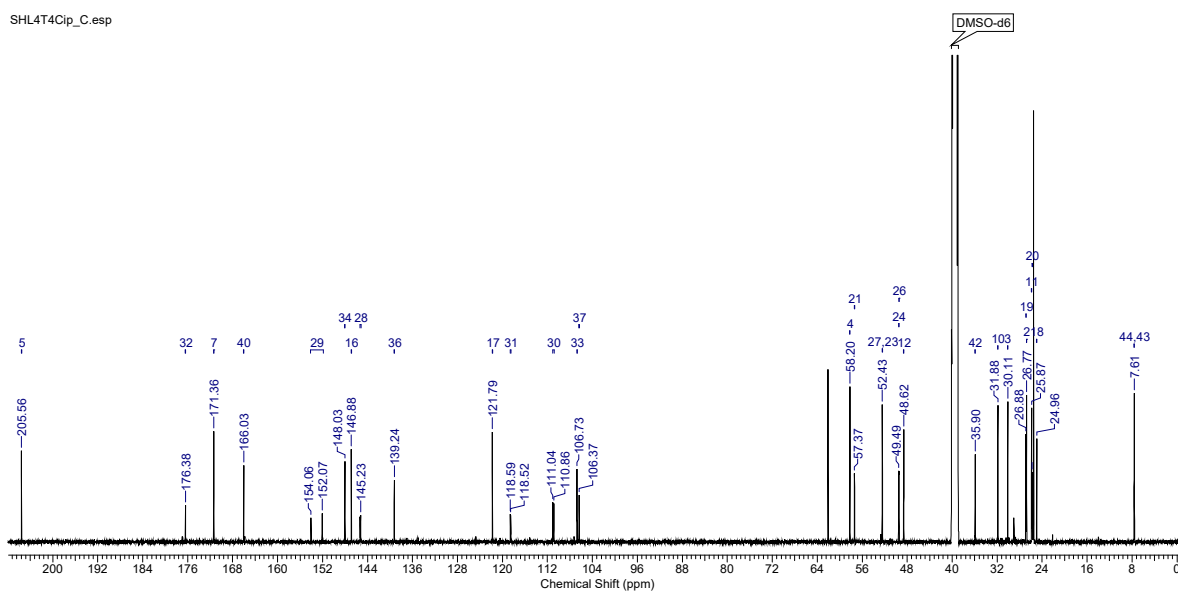
10.19 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 156



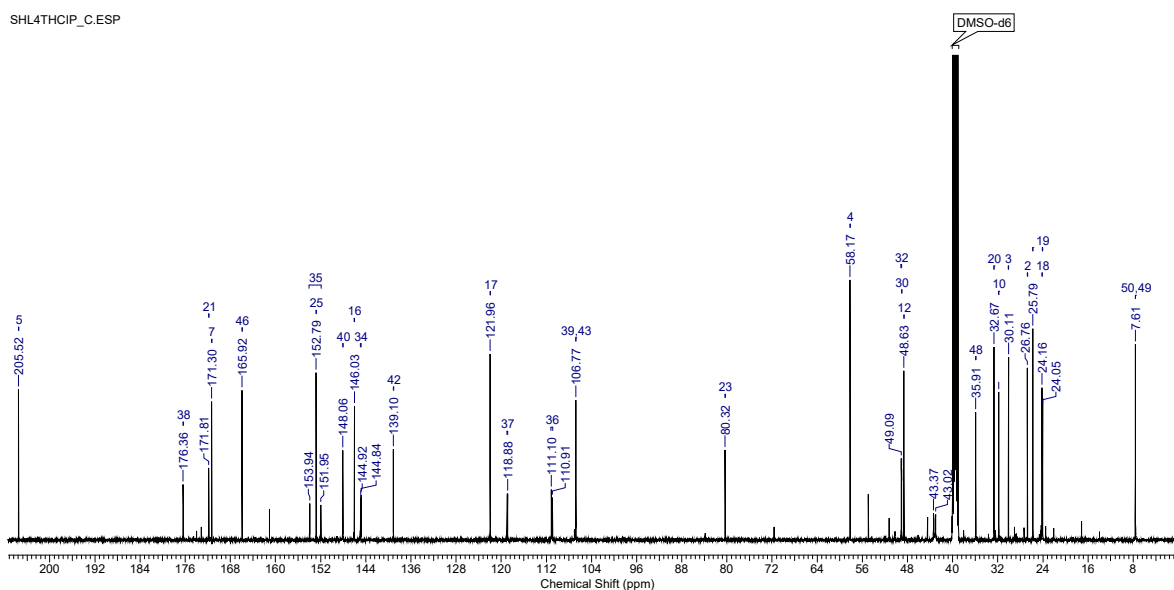
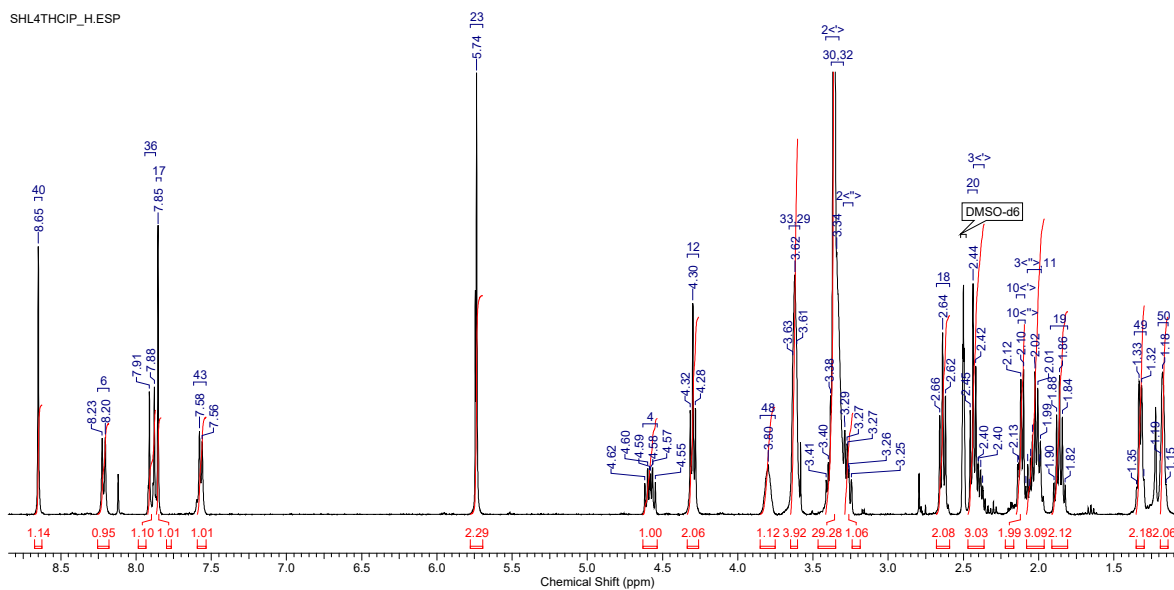
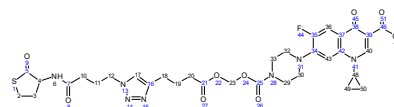
SHL4T4Cip\_H.esp



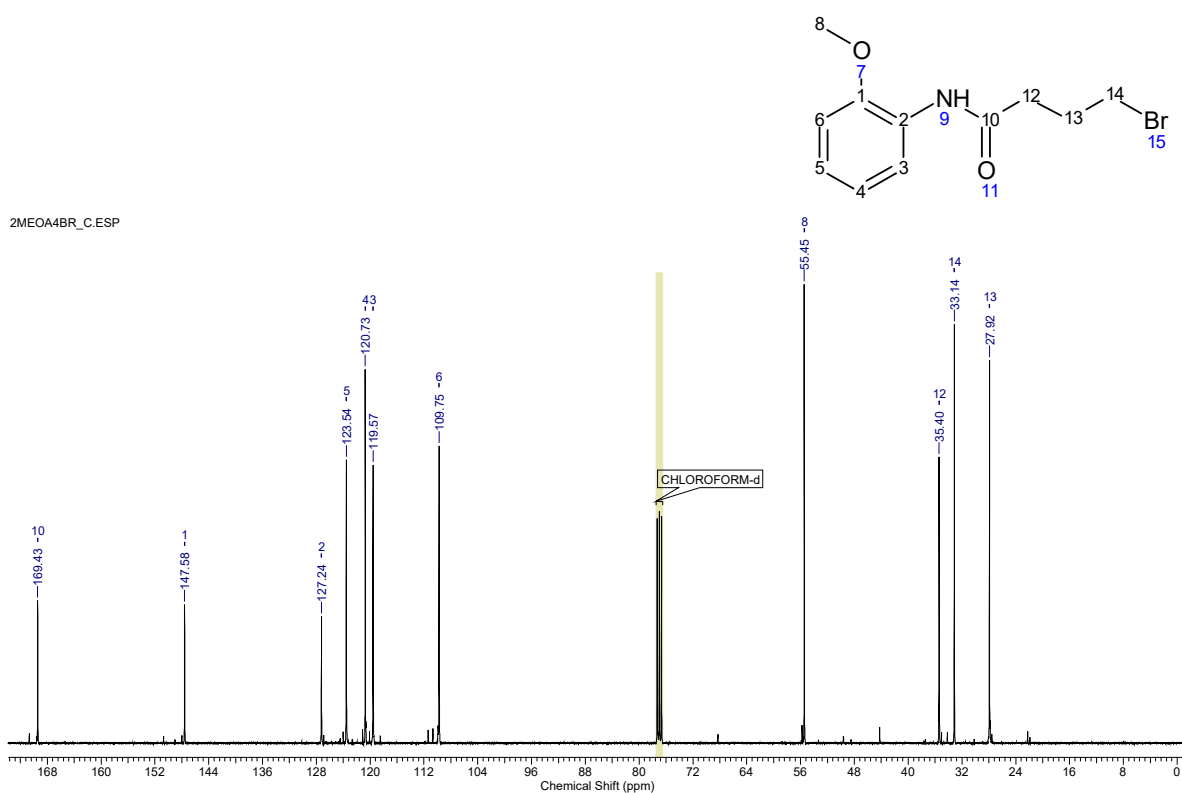
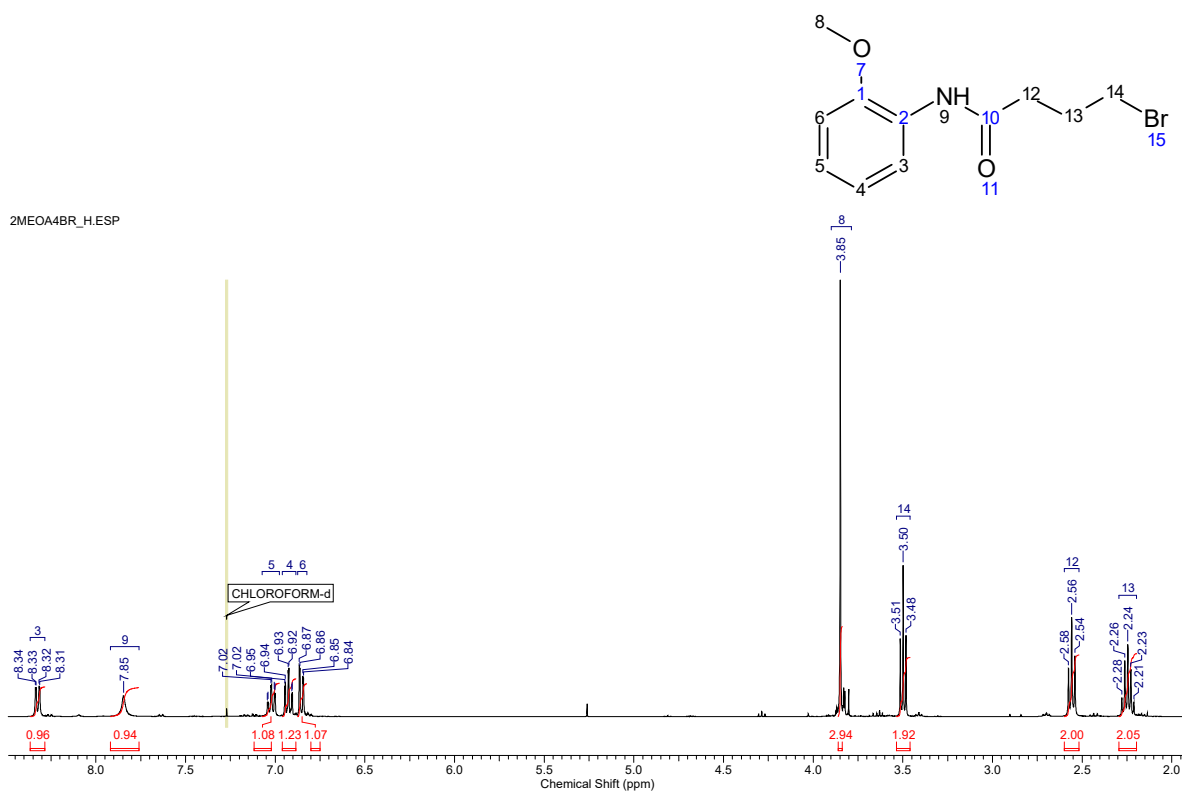
SHL4T4Cip\_C.esp



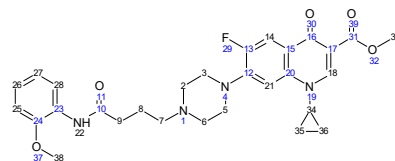
10.20 1-Cyclopropyl-6-fluoro-4-oxo-7-(4((((4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 157



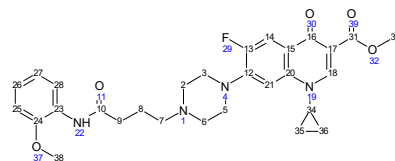
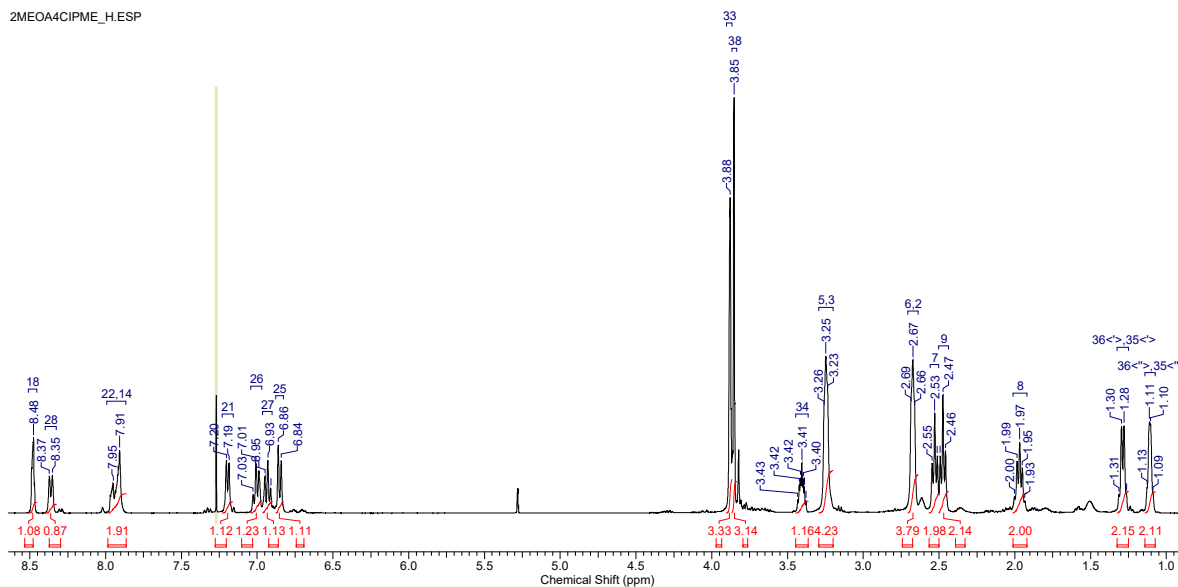
# 10.21 4-Bromo-*N*-(2-methoxyphenyl)butanamide 159



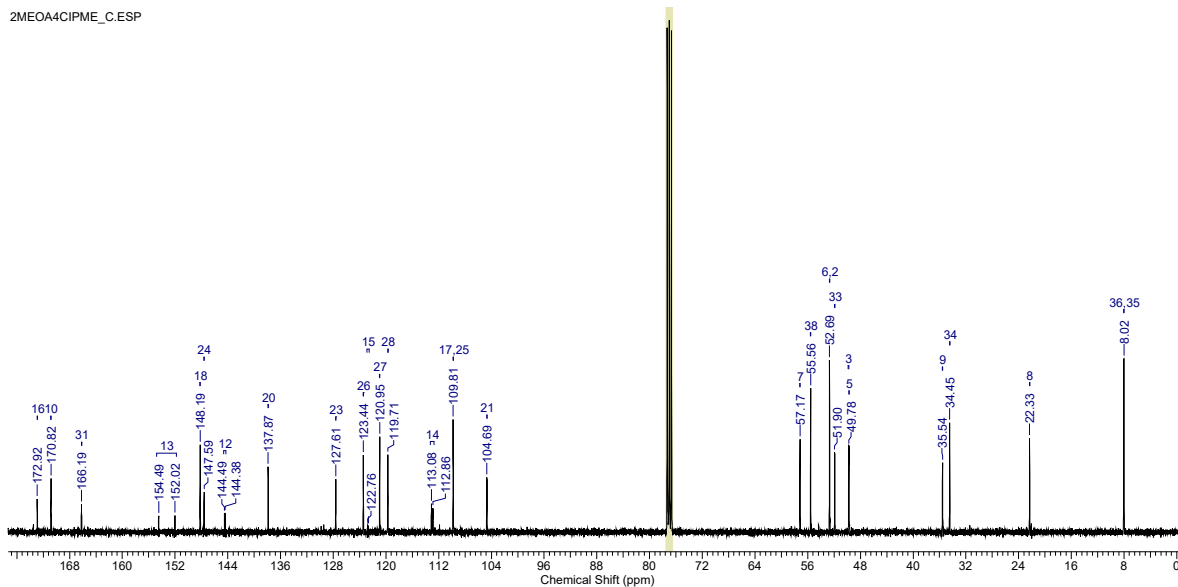
10.22 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 160



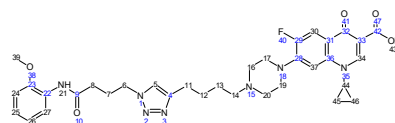
2MEOA4CIPME\_H.ESP



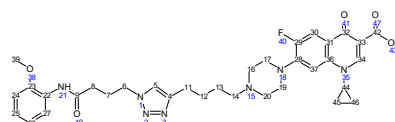
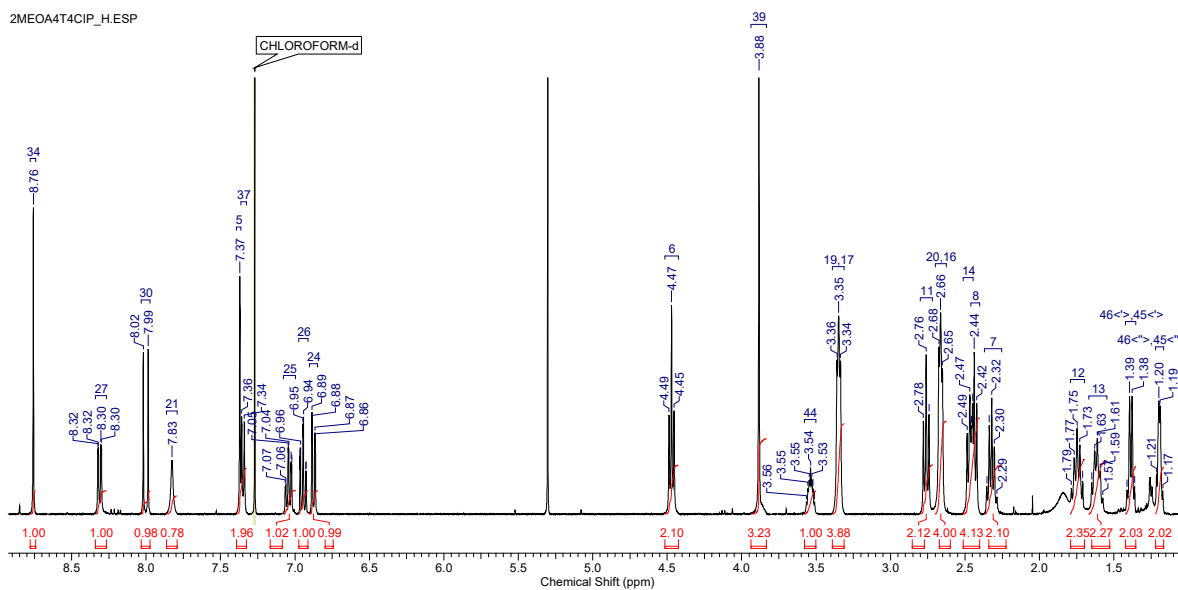
2MEOA4CIPME\_C.ESP



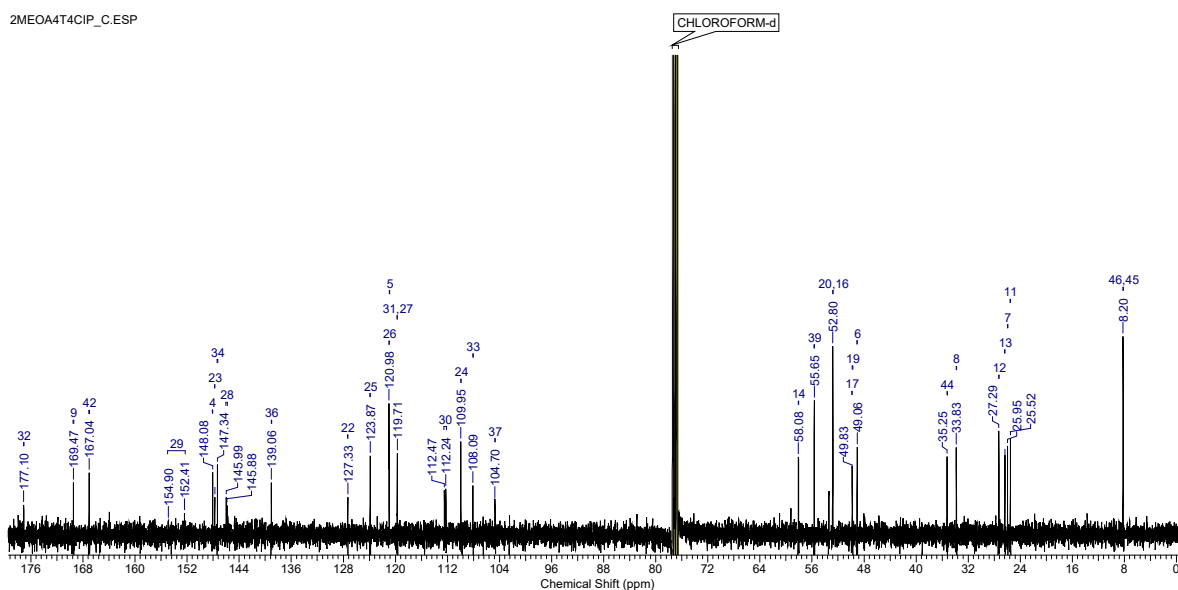
10.23 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 162



2MEOA4T4CIP\_H.ESP

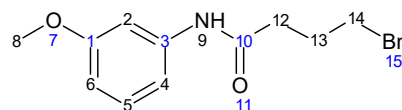


2MEOA4T4CIP\_C.ESP

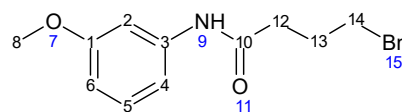
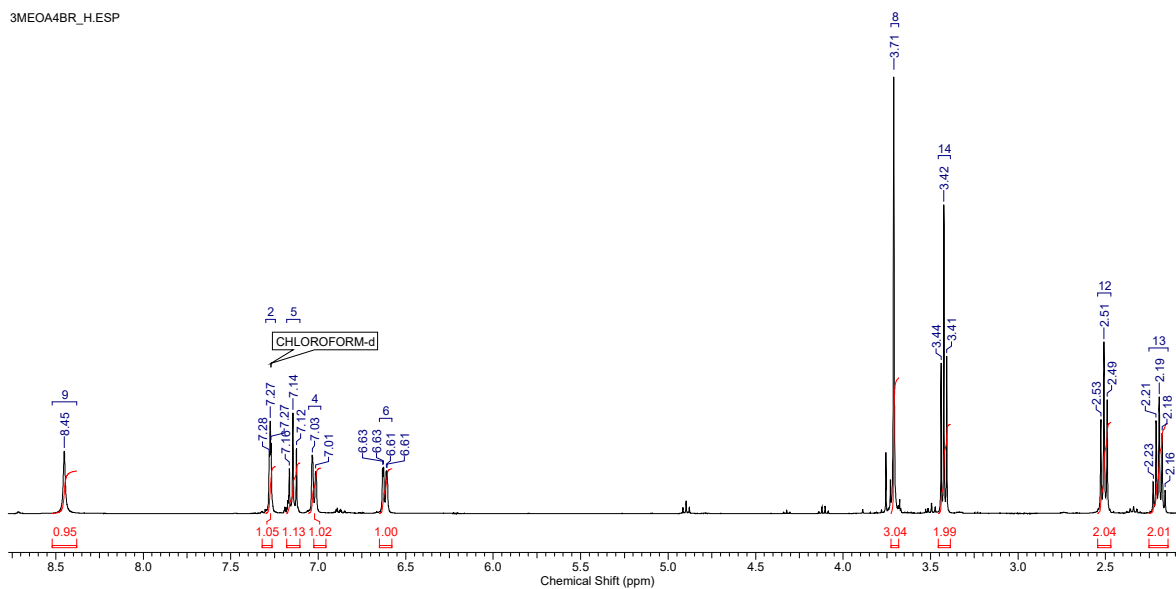




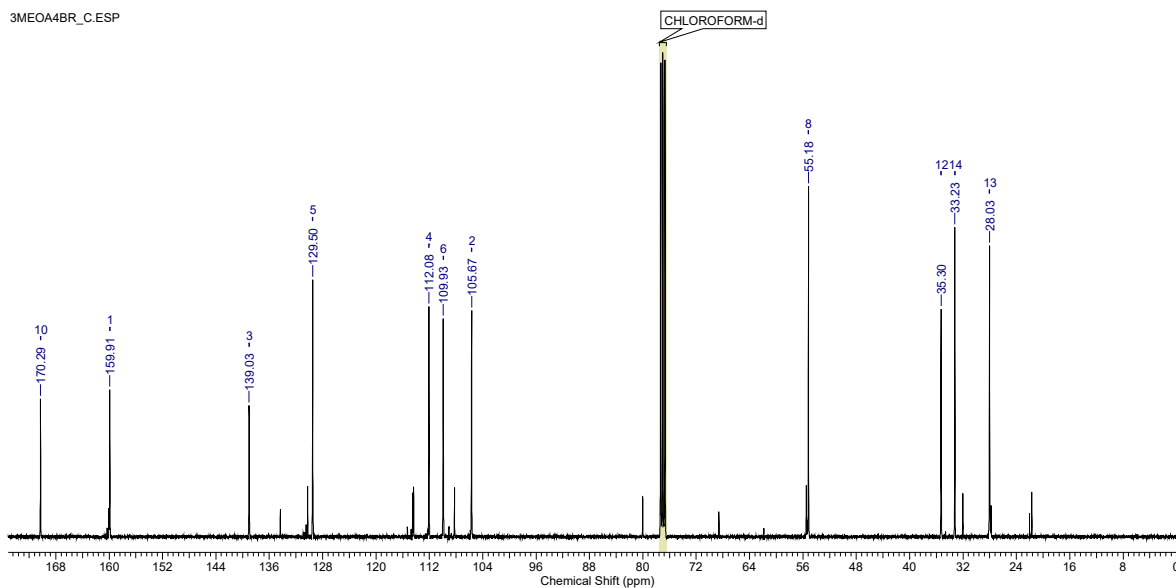
## 10.24 4-Bromo-*N*-(3-methoxyphenyl)butanamide 164



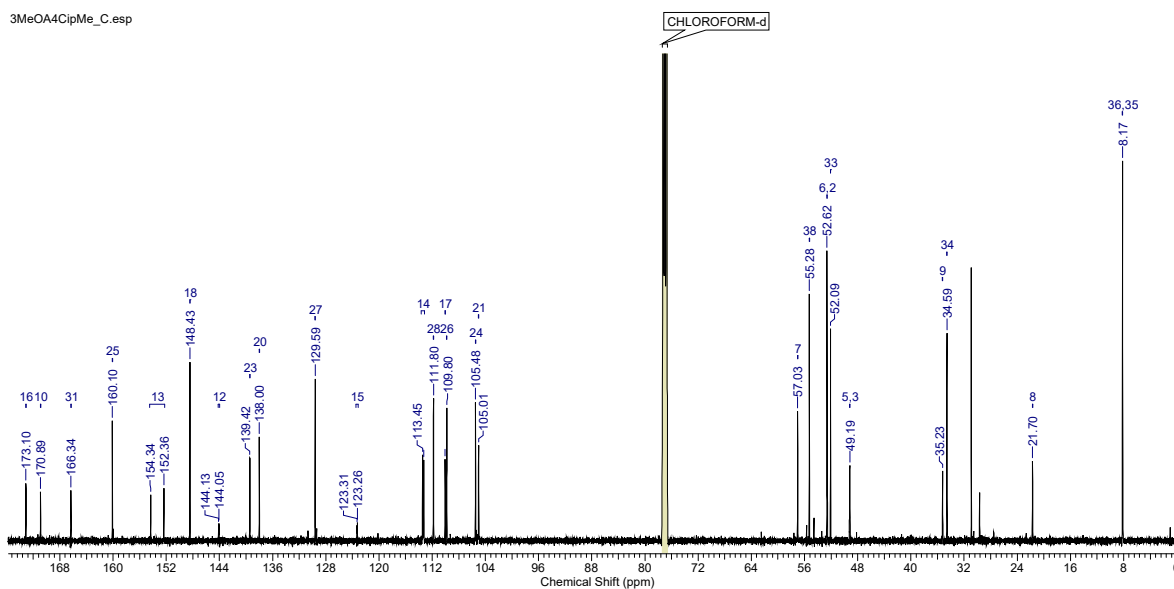
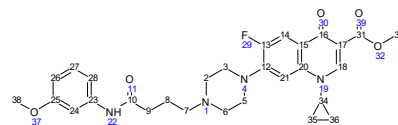
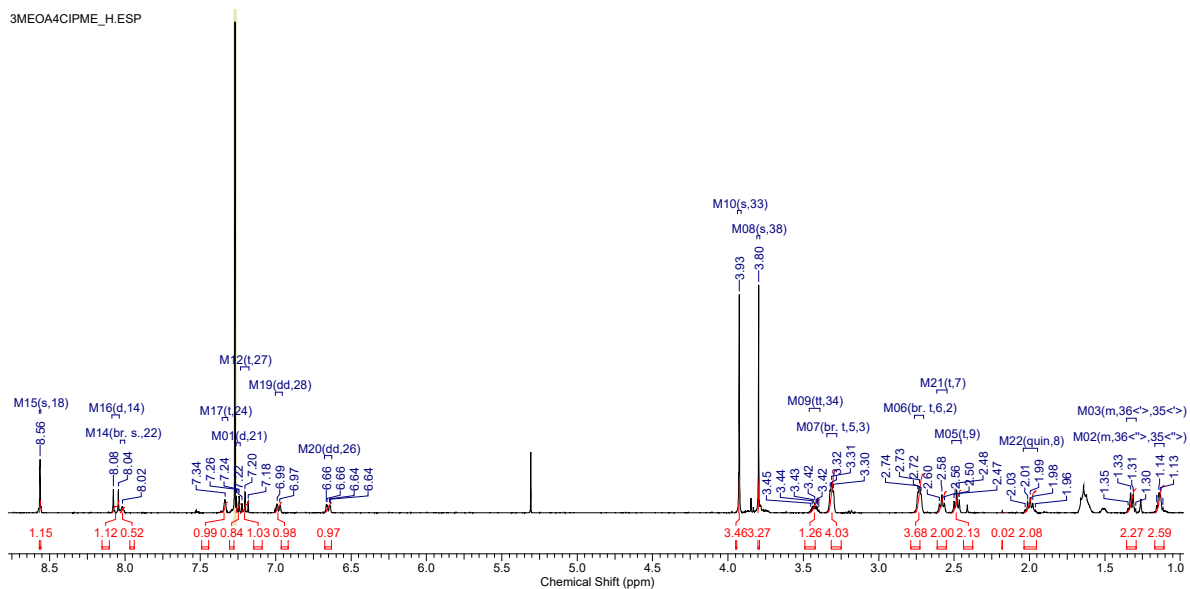
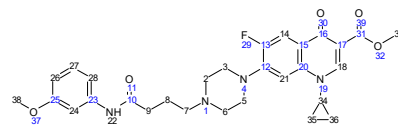
3MEOA4BR\_H.ESP



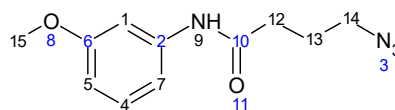
3MEOA4BR\_C.ESP



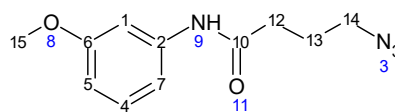
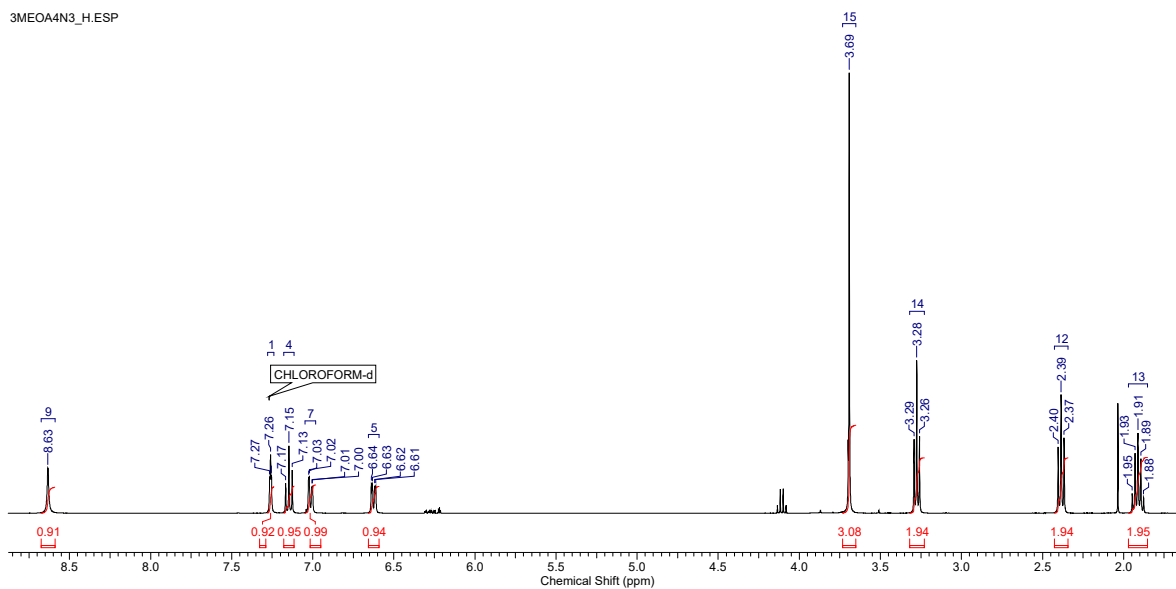
10.25 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 165



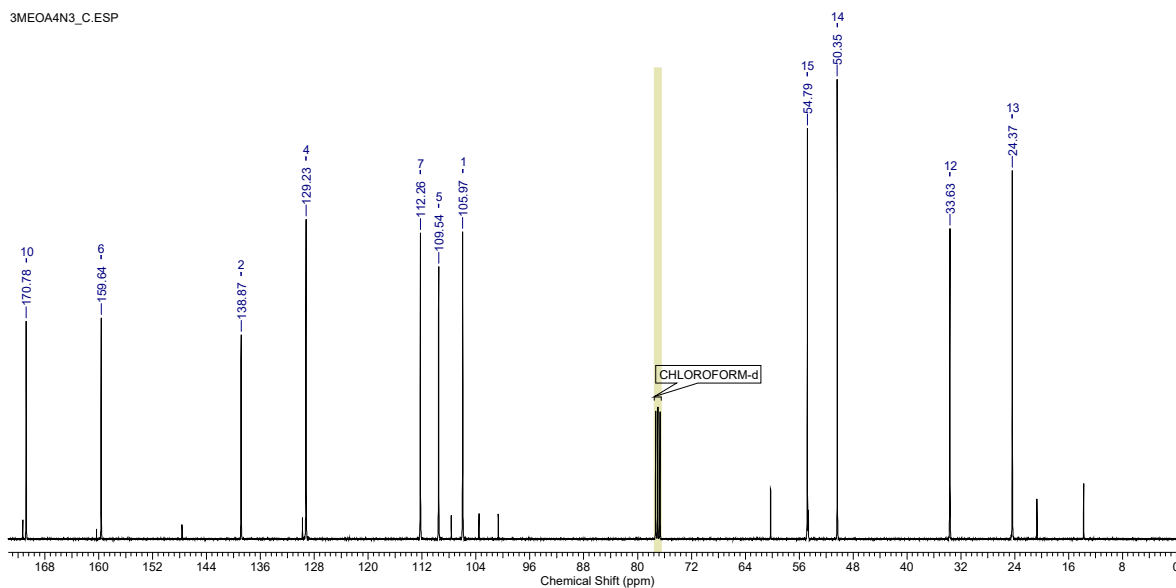
## 10.26 4-Azido-*N*-(3-methoxyphenyl)butanamide 166



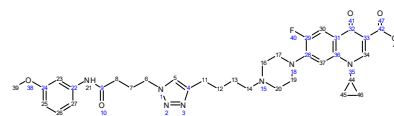
3MEOA4N3\_H.ESP



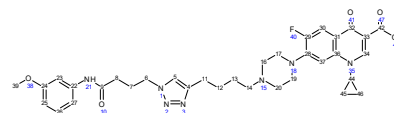
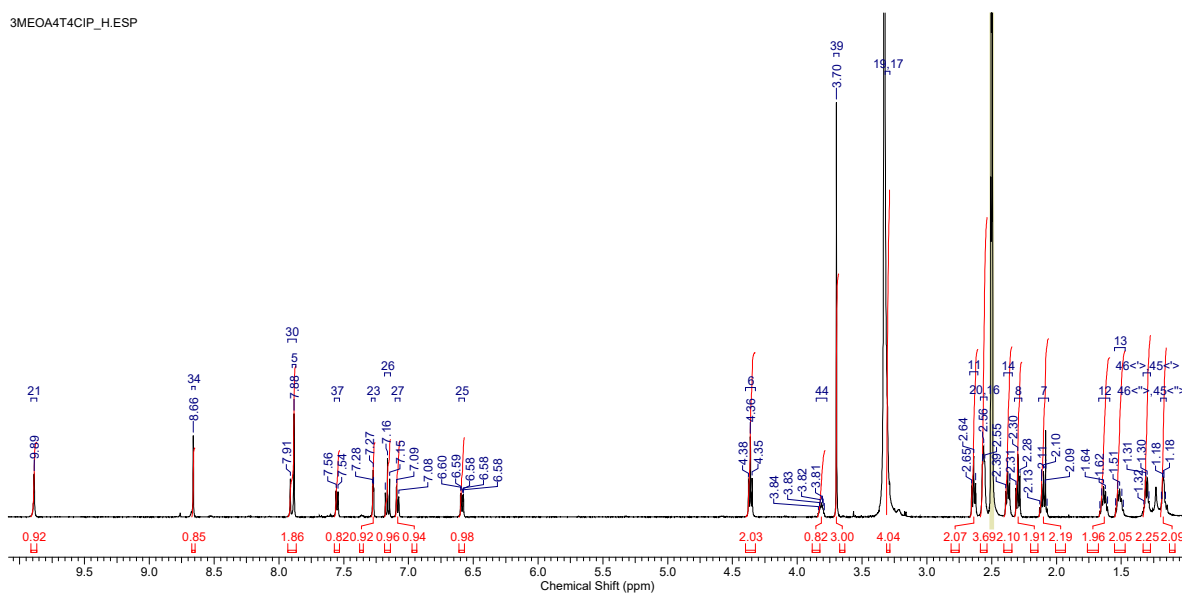
3MEOA4N3\_C.ESP



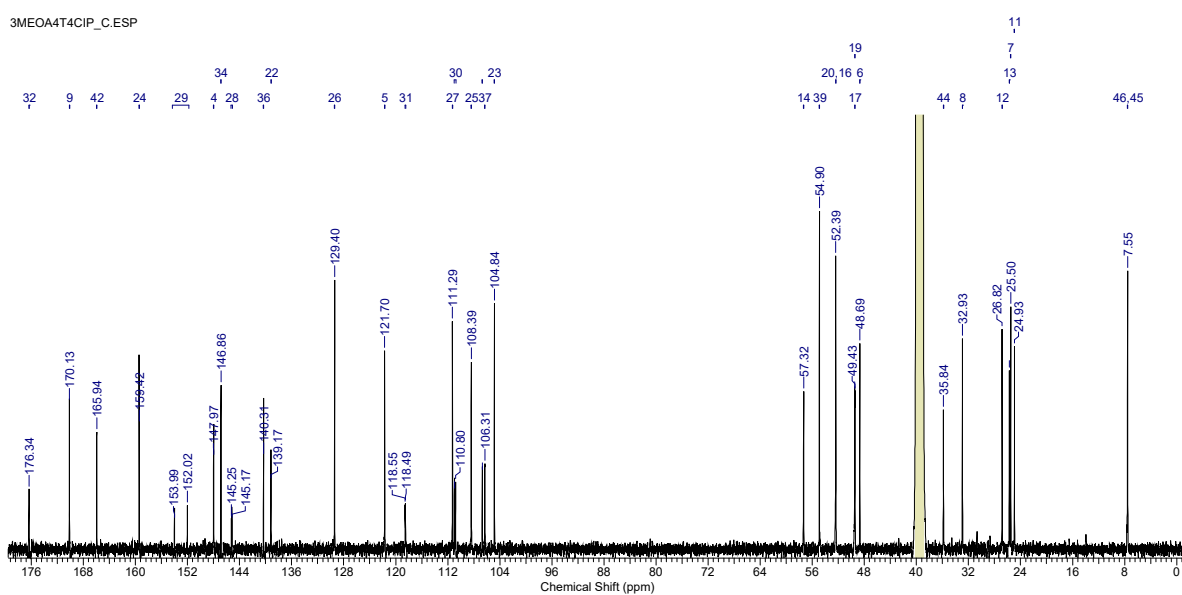
10.27 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 167



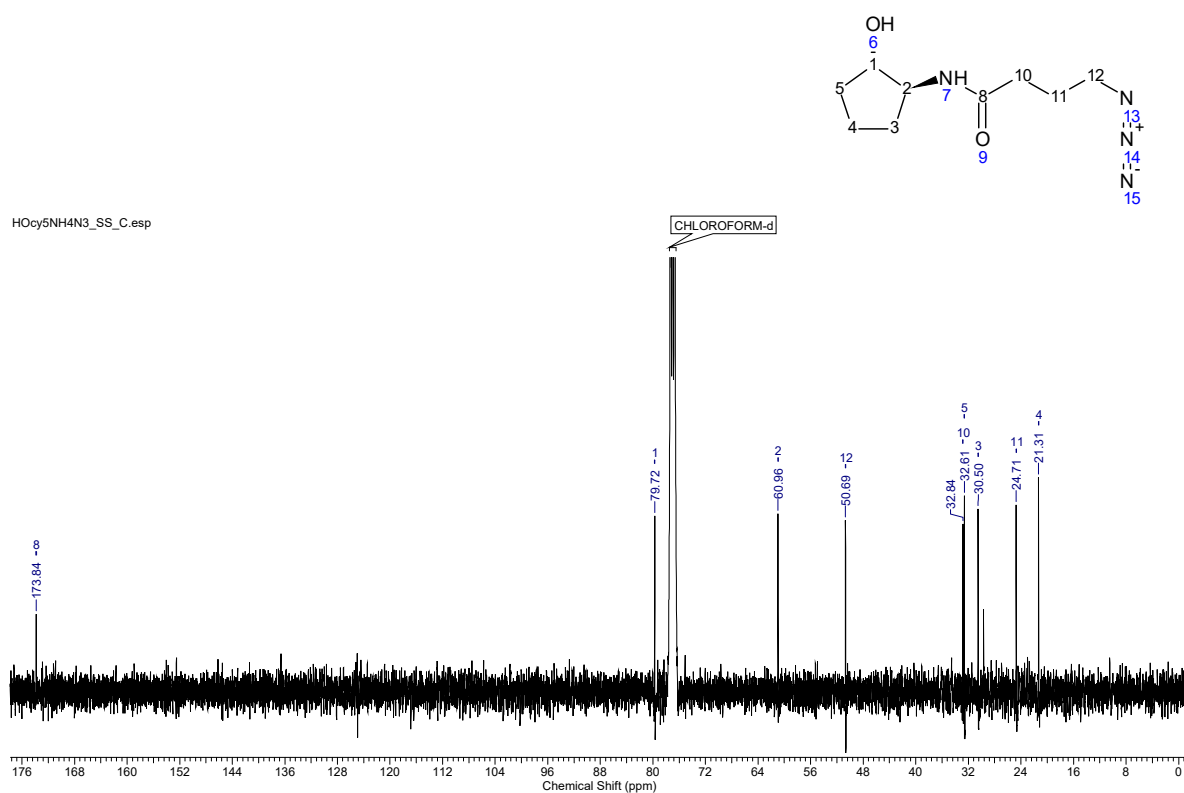
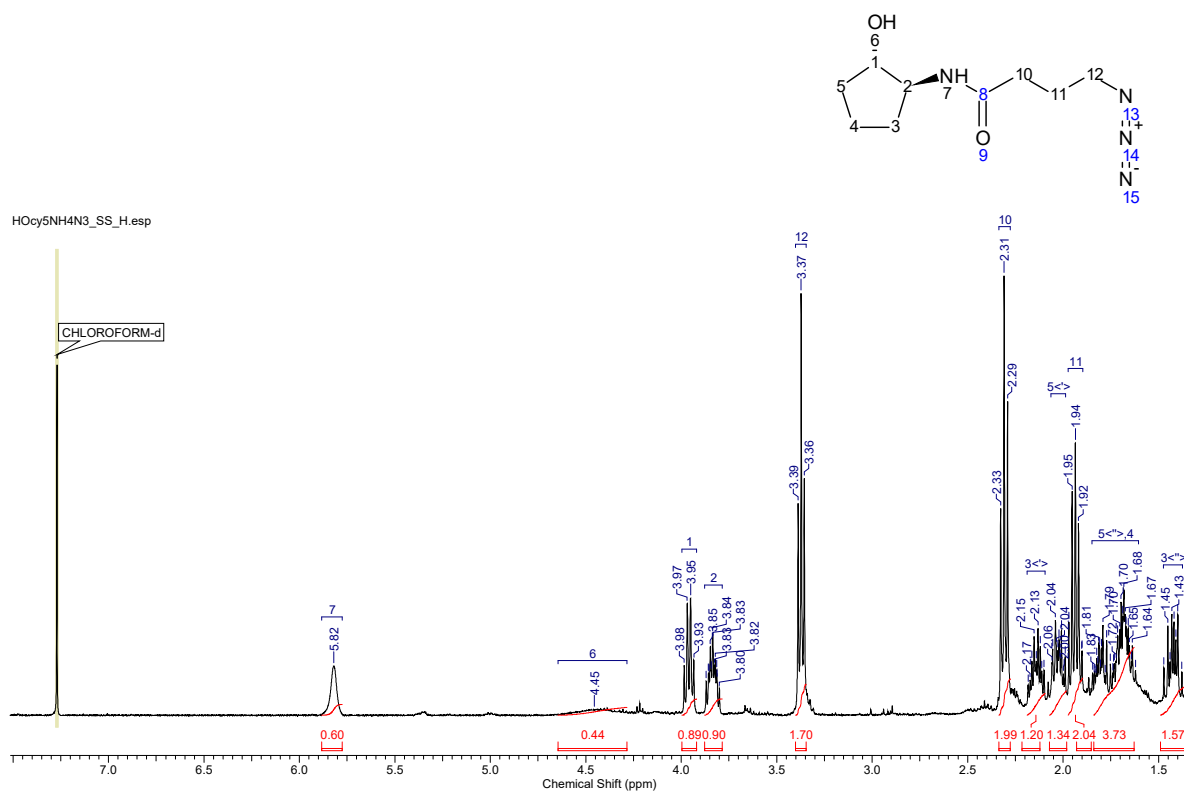
3MEOA4T4CIP\_H.ESP



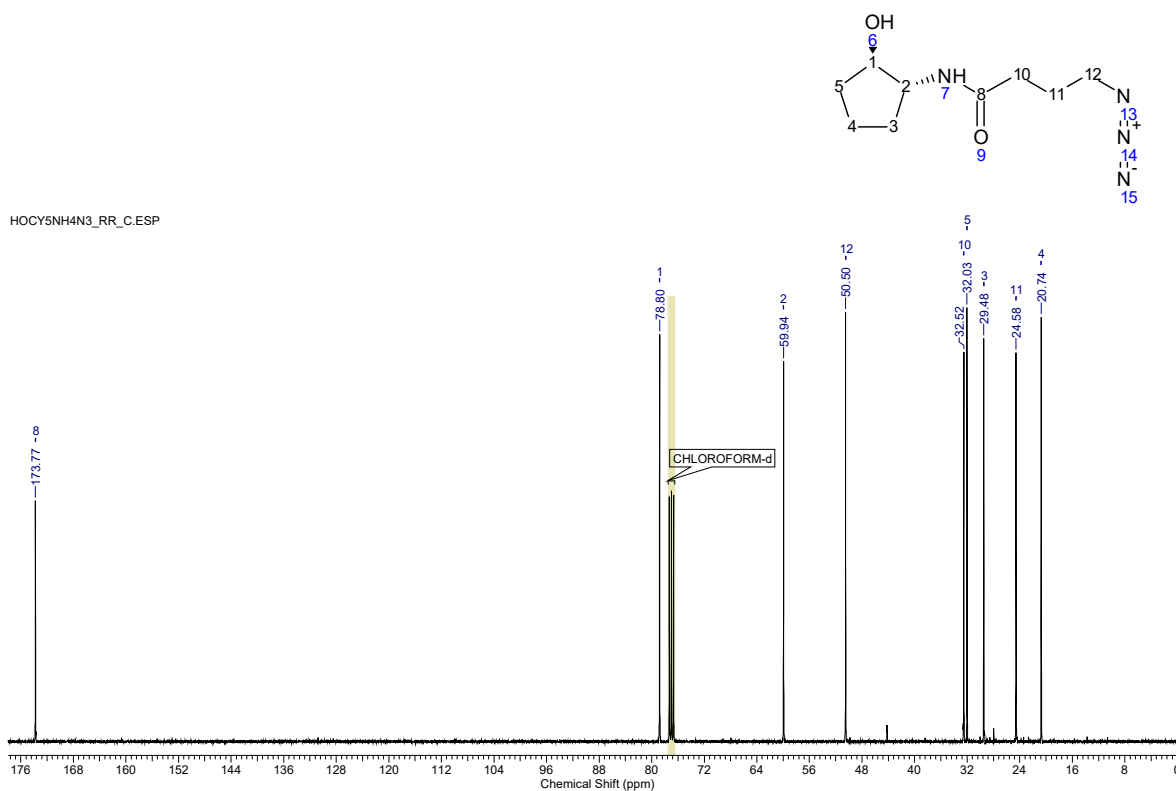
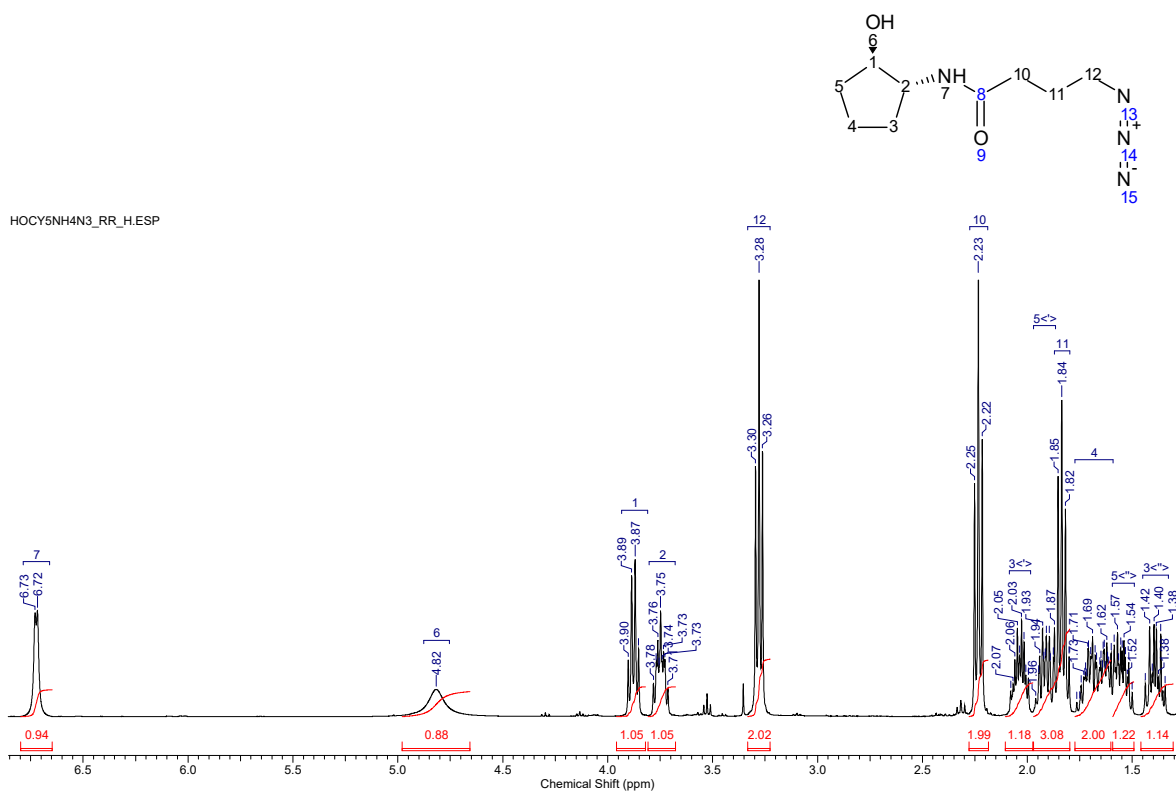
3MEOA4T4CIP\_C.ESP



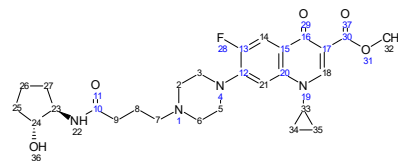
10.28 4-Azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide 176



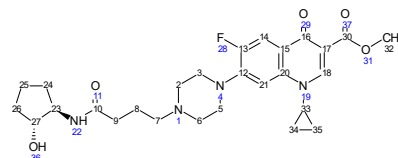
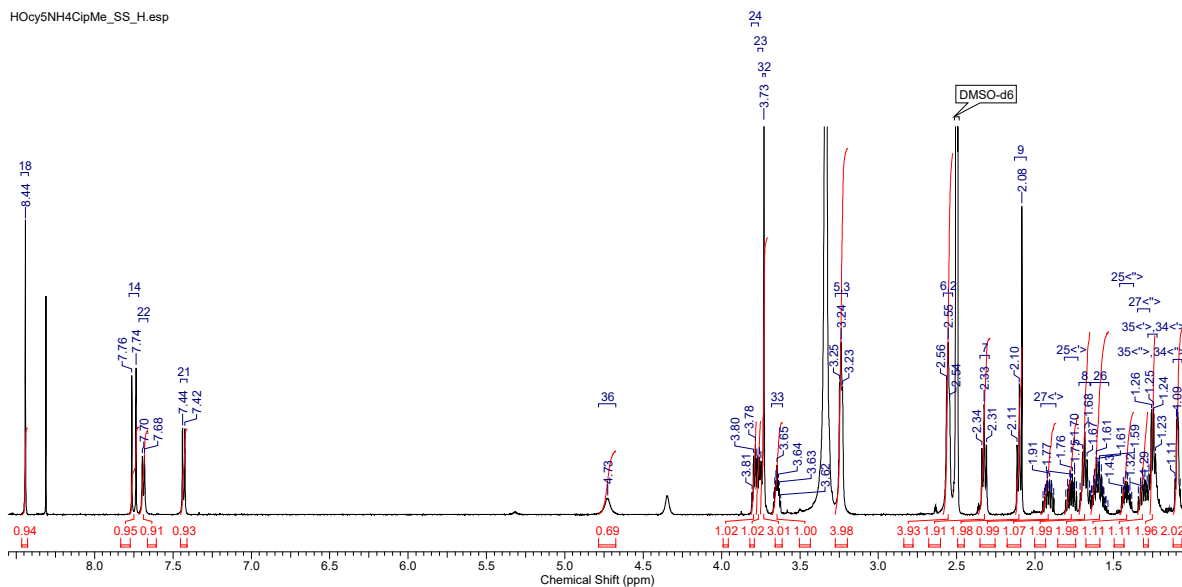
# 10.29 4-Azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide 177



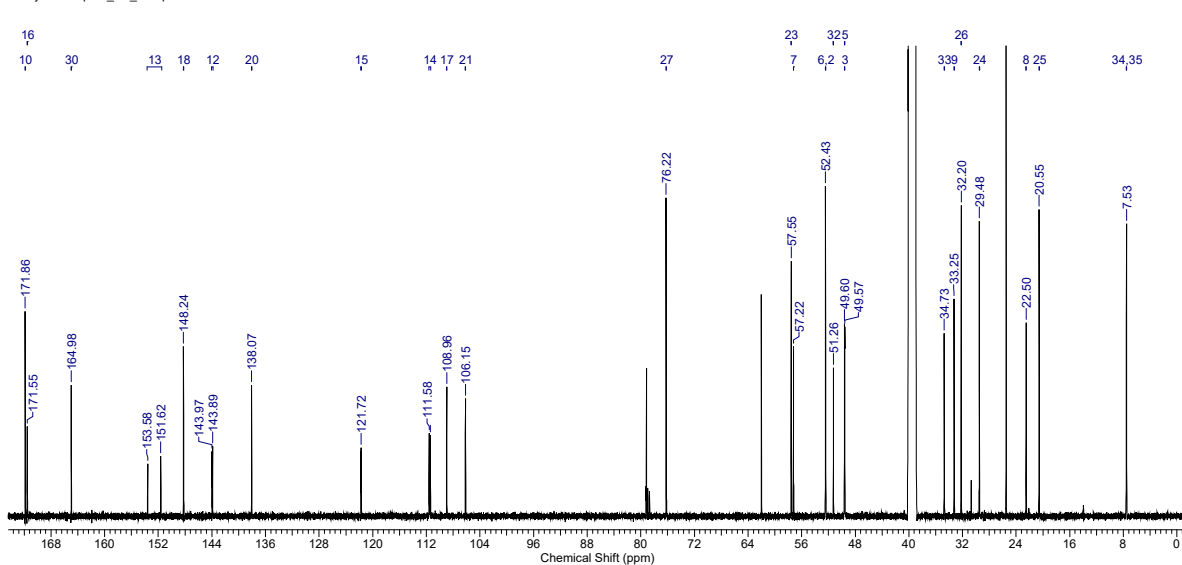
10.30 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 178



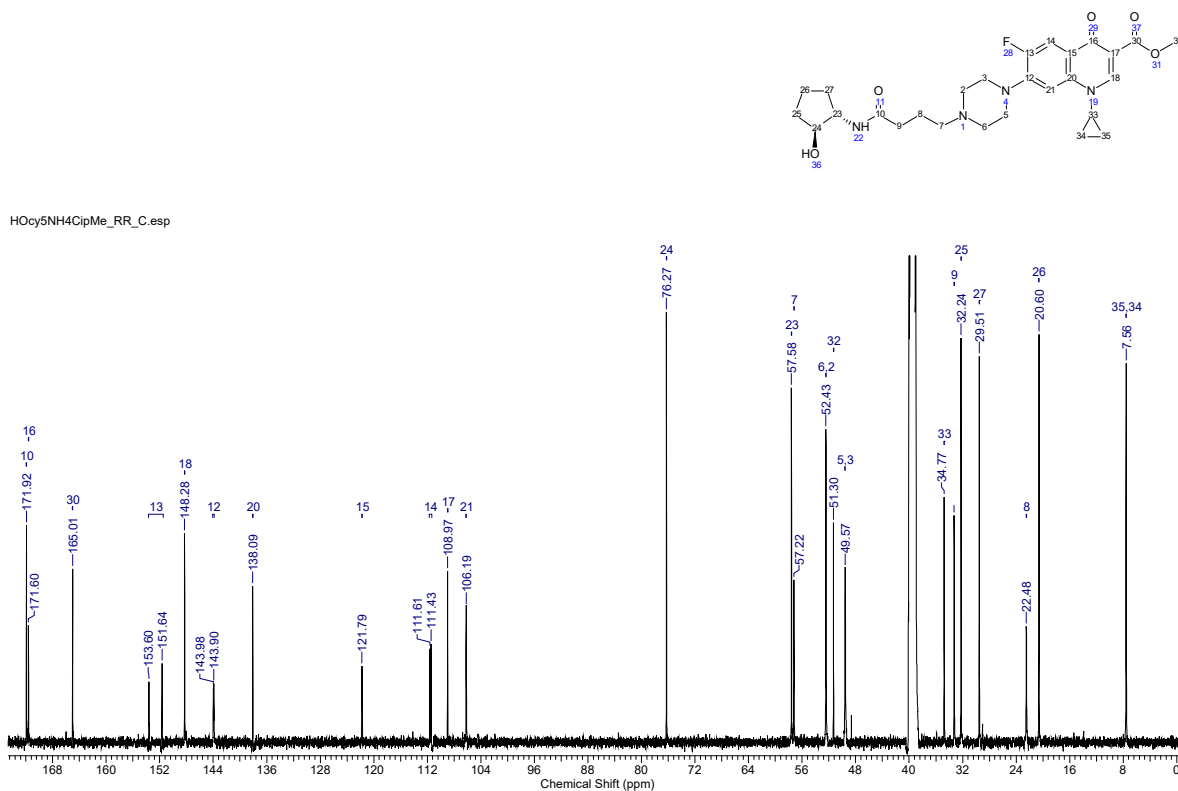
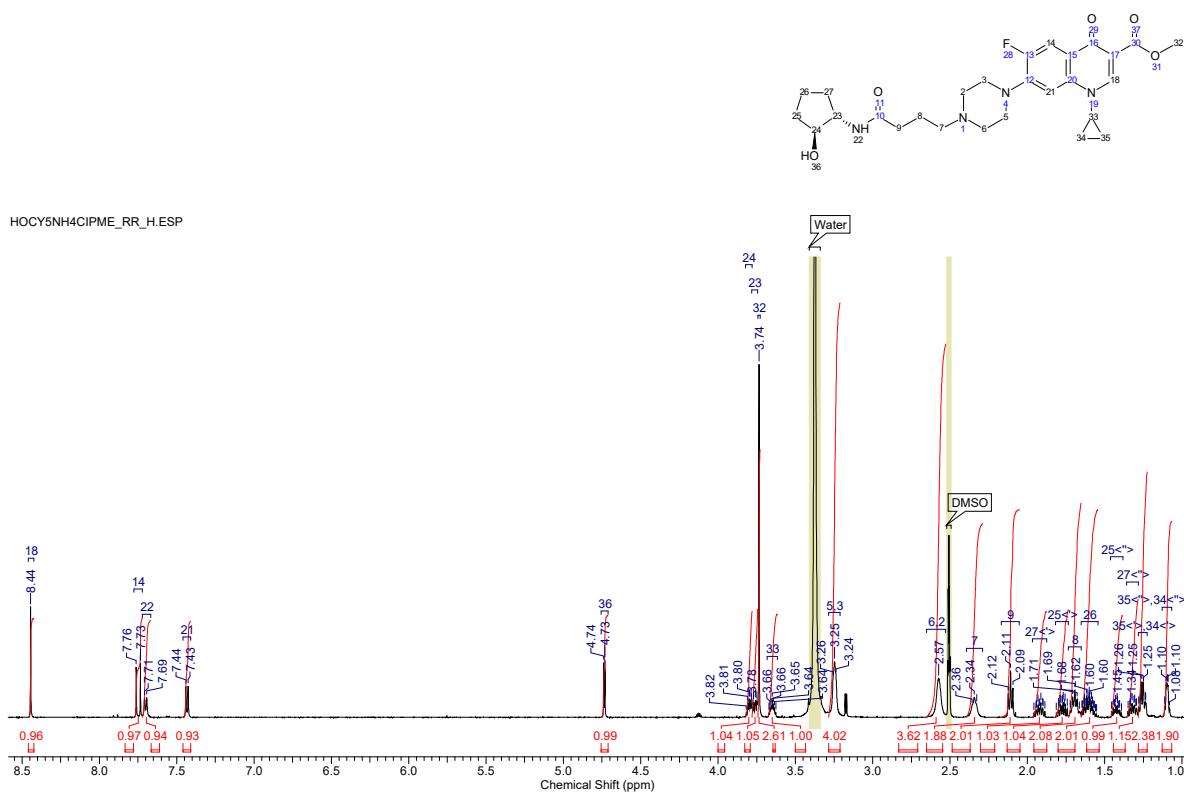
HOcy5NH4CipMe\_SS\_H.esp



HOcy5NH4CipMe\_SS\_C.esp

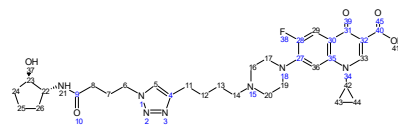


10.31 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 179

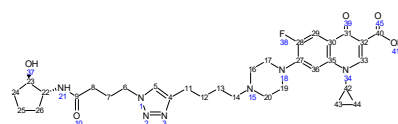
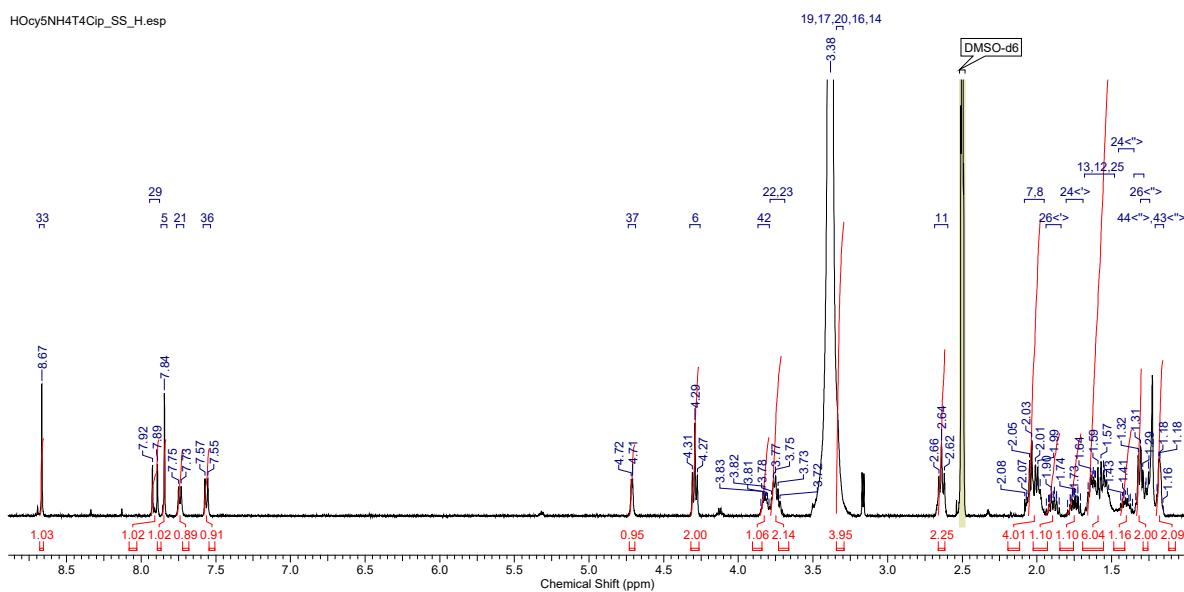




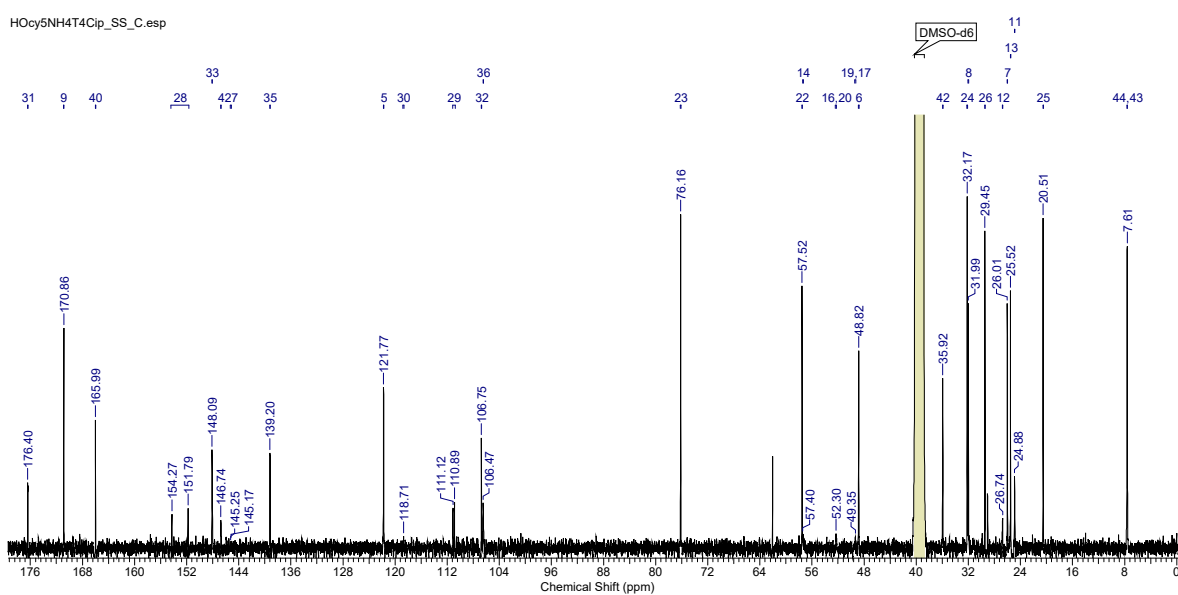
10.32 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 180



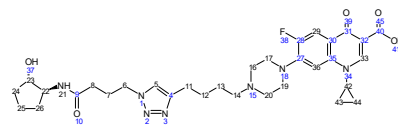
HOcy5NH4T4Cip\_SS\_H.esp



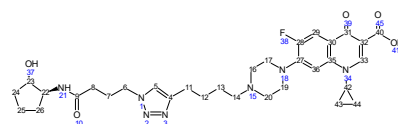
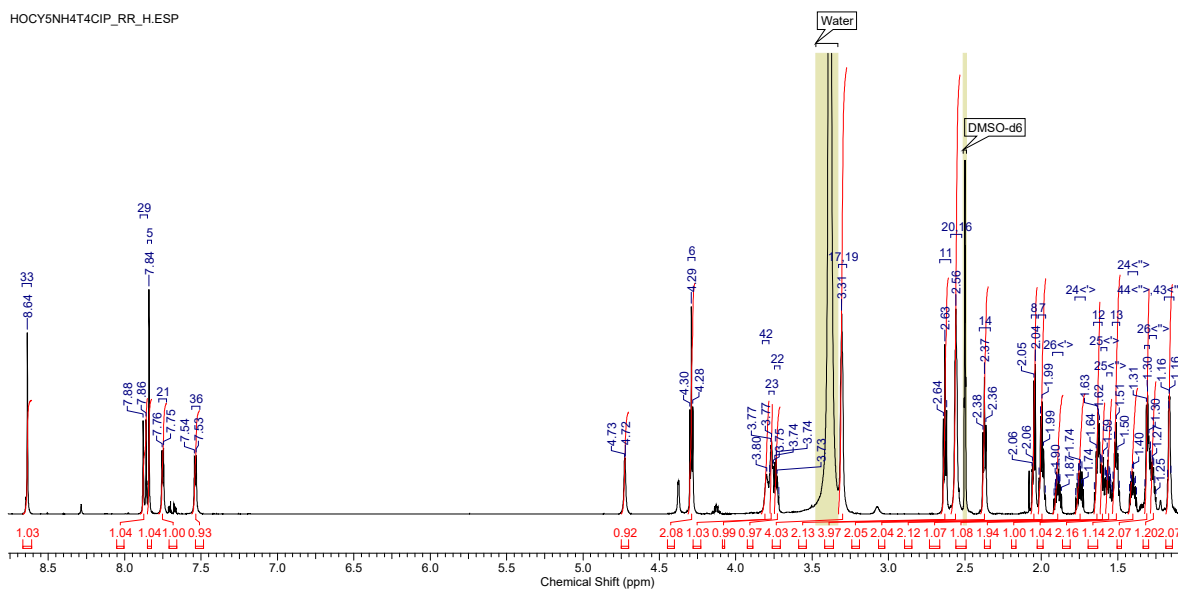
HOcy5NH4T4Cip\_SS\_C.esp



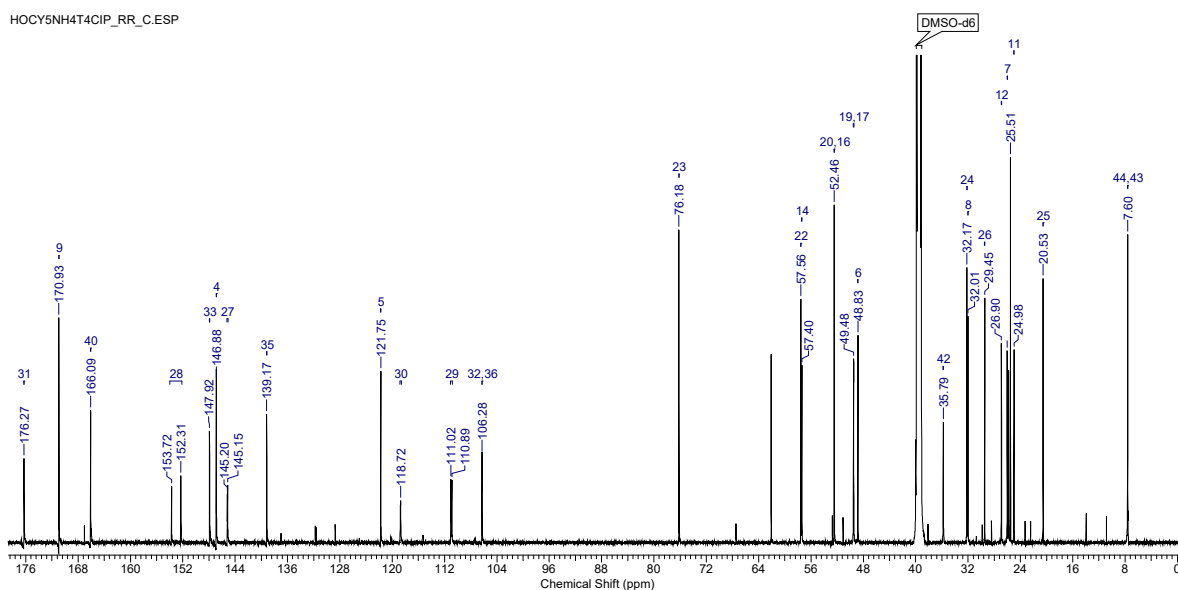
10.33 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 181



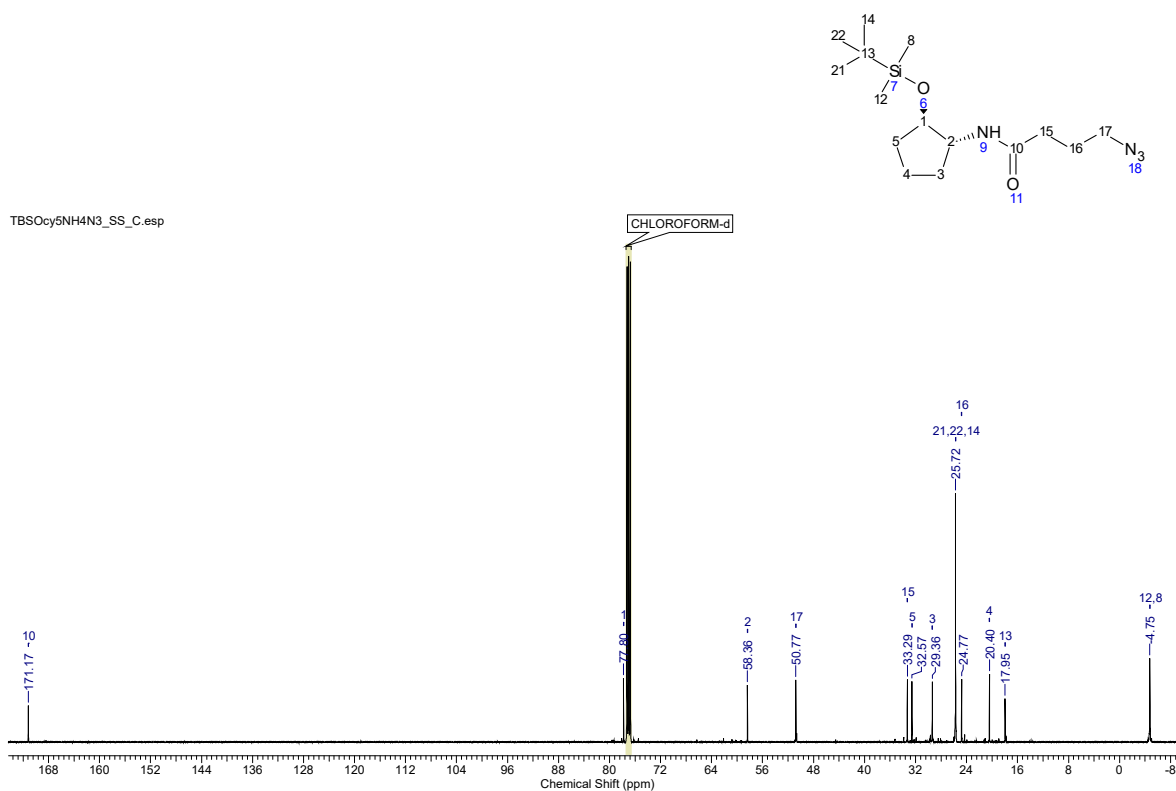
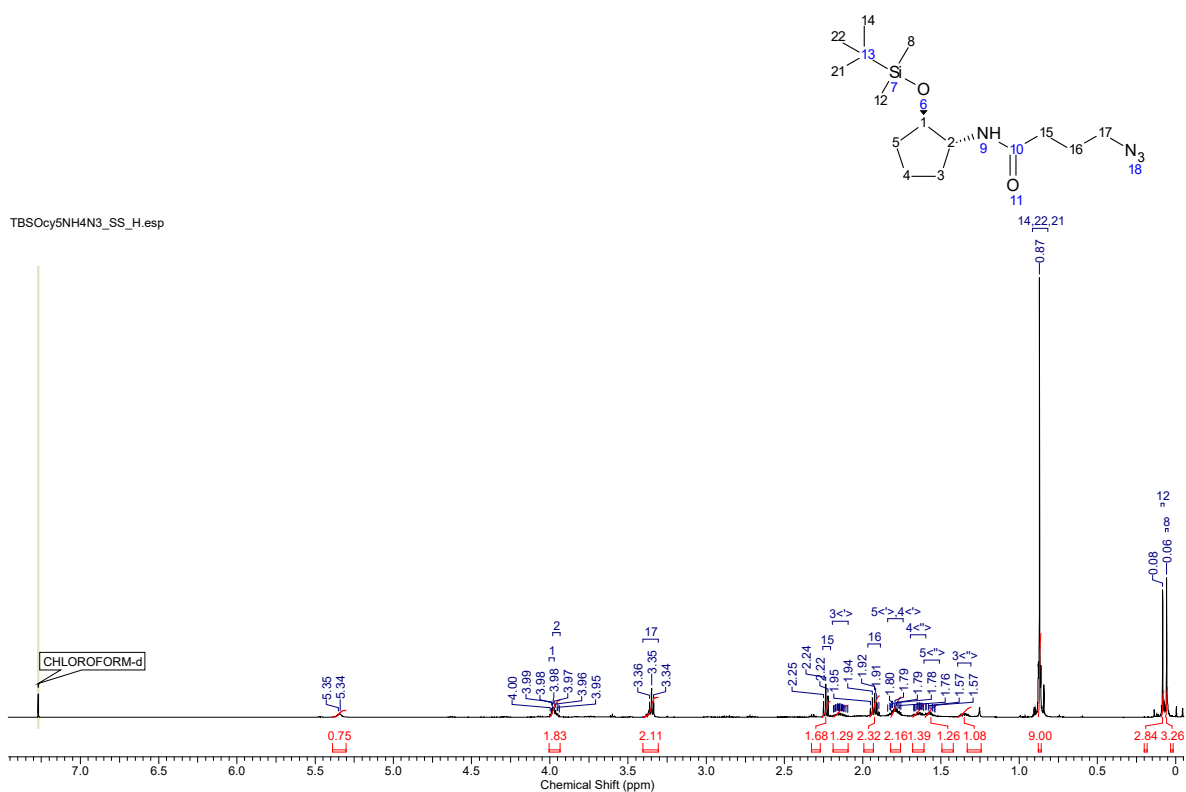
HOCY5NH4T4CIP\_RR\_H.ESP



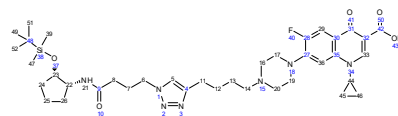
HOCY5NH4T4CIP\_RR\_C.ESP



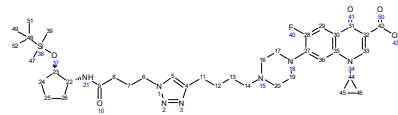
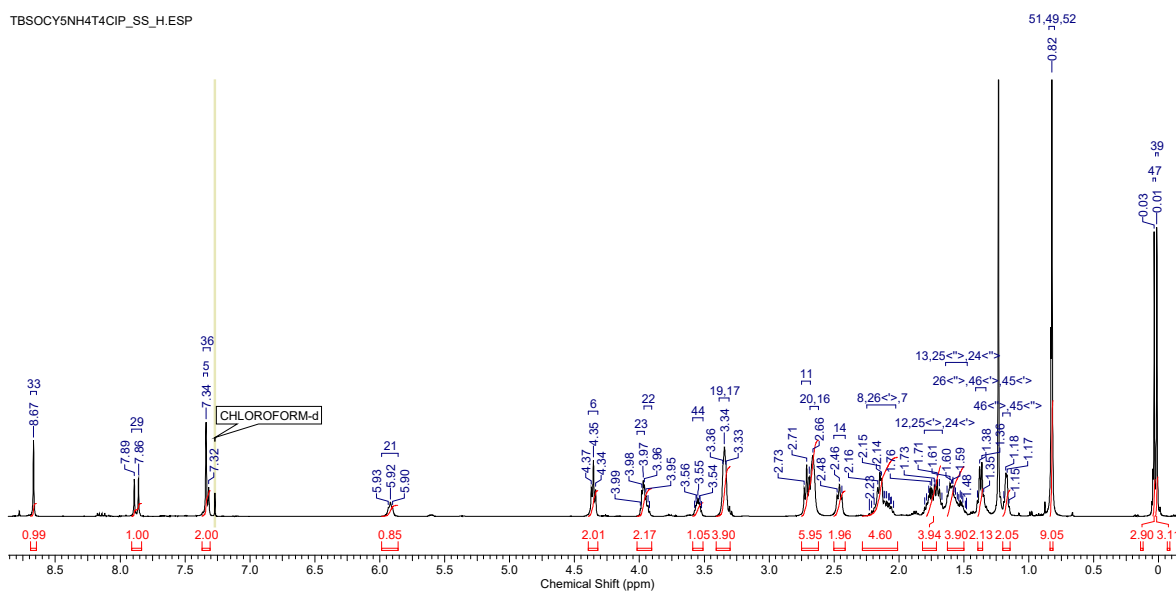
10.34 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide  
186



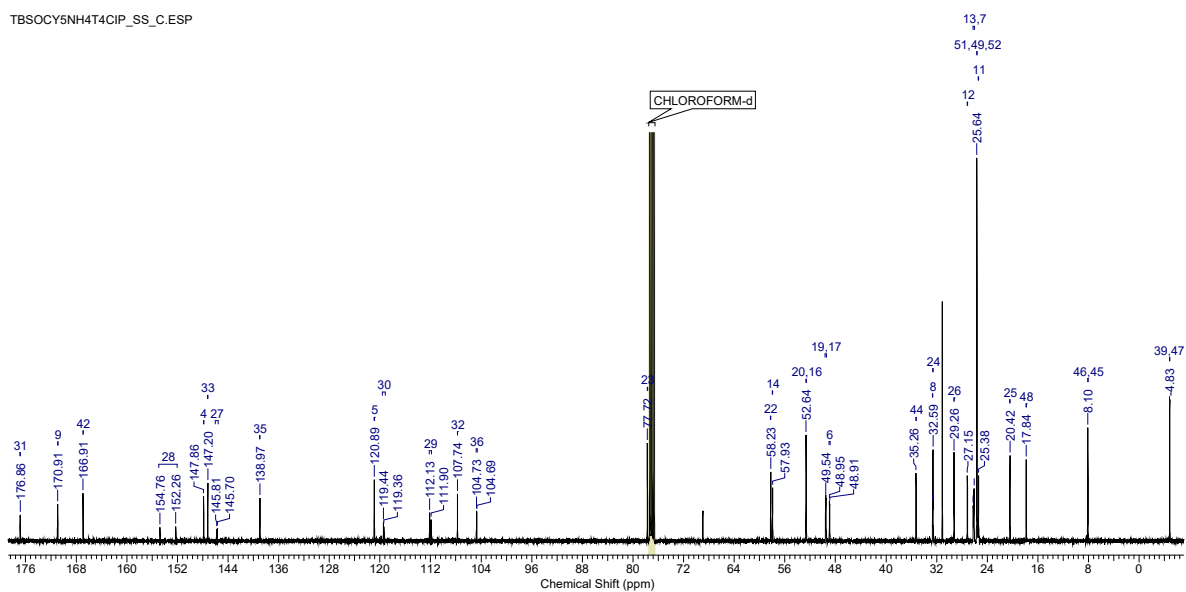
10.35 7-(4-(4-(1-(4-(((1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190



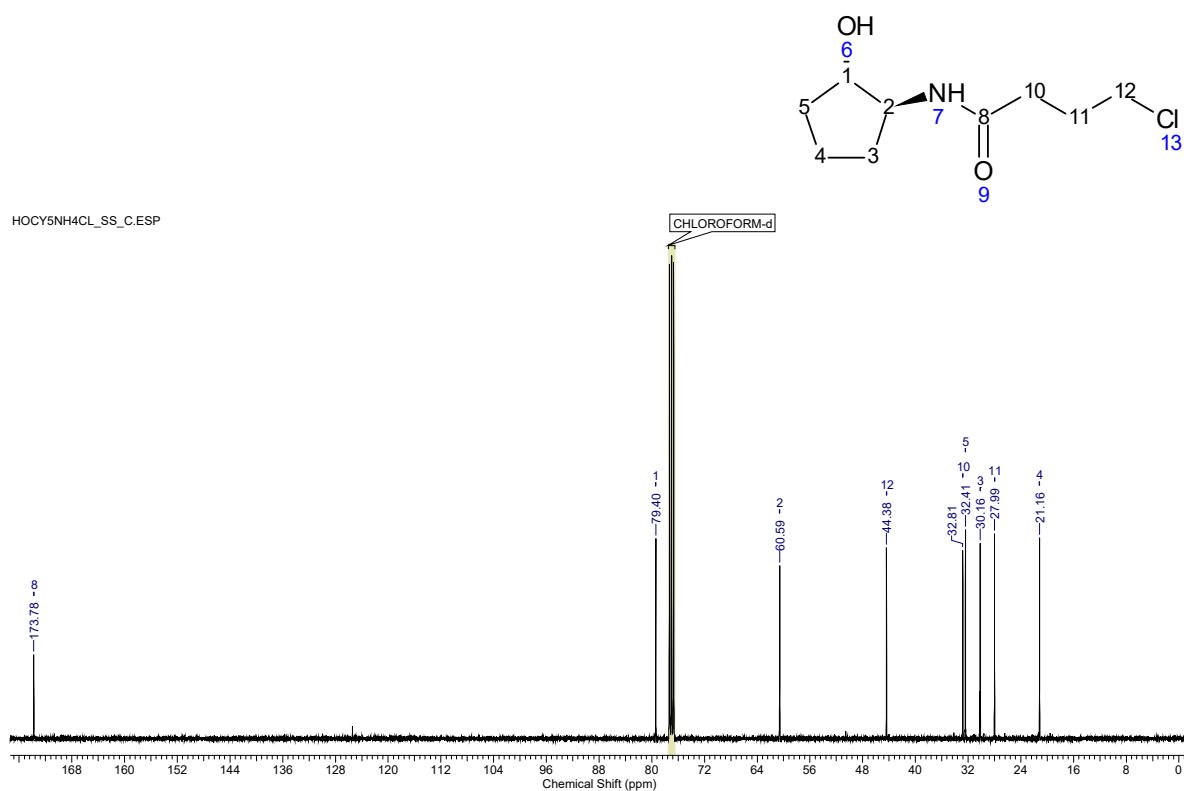
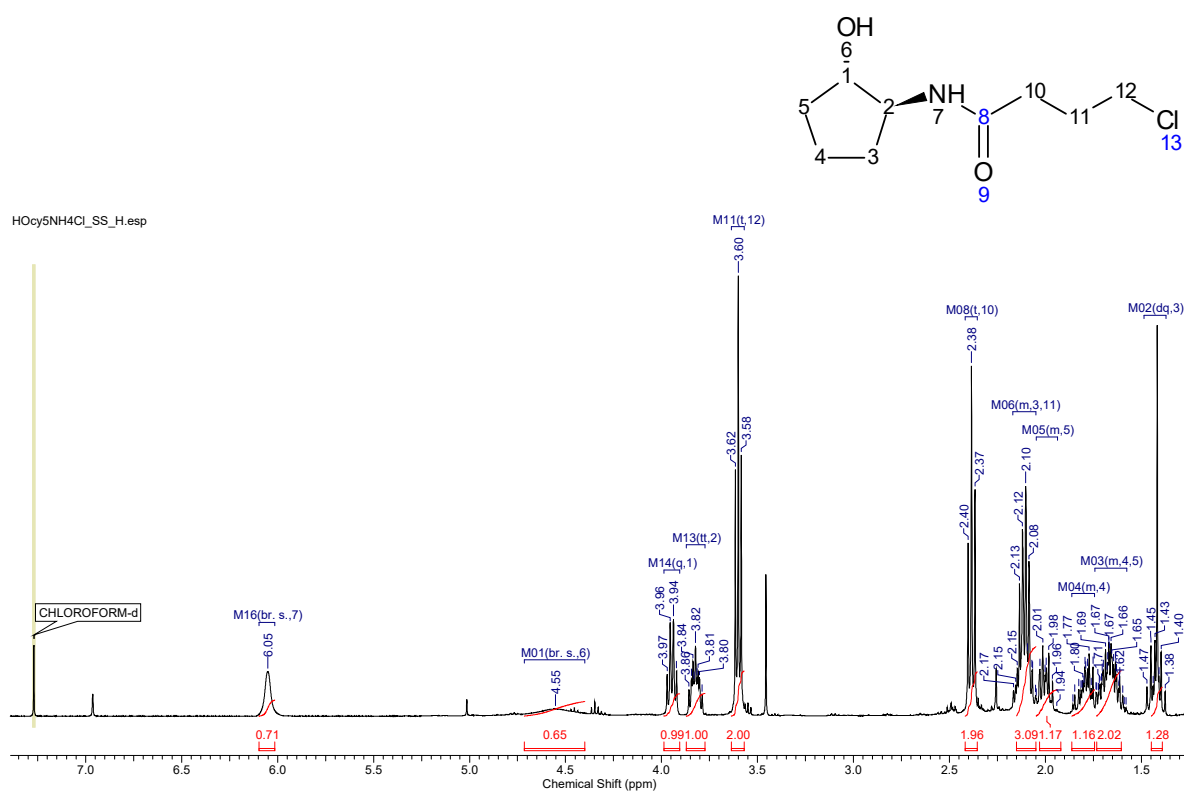
TBSOCY5NH4T4CIP\_SS\_H.ESP



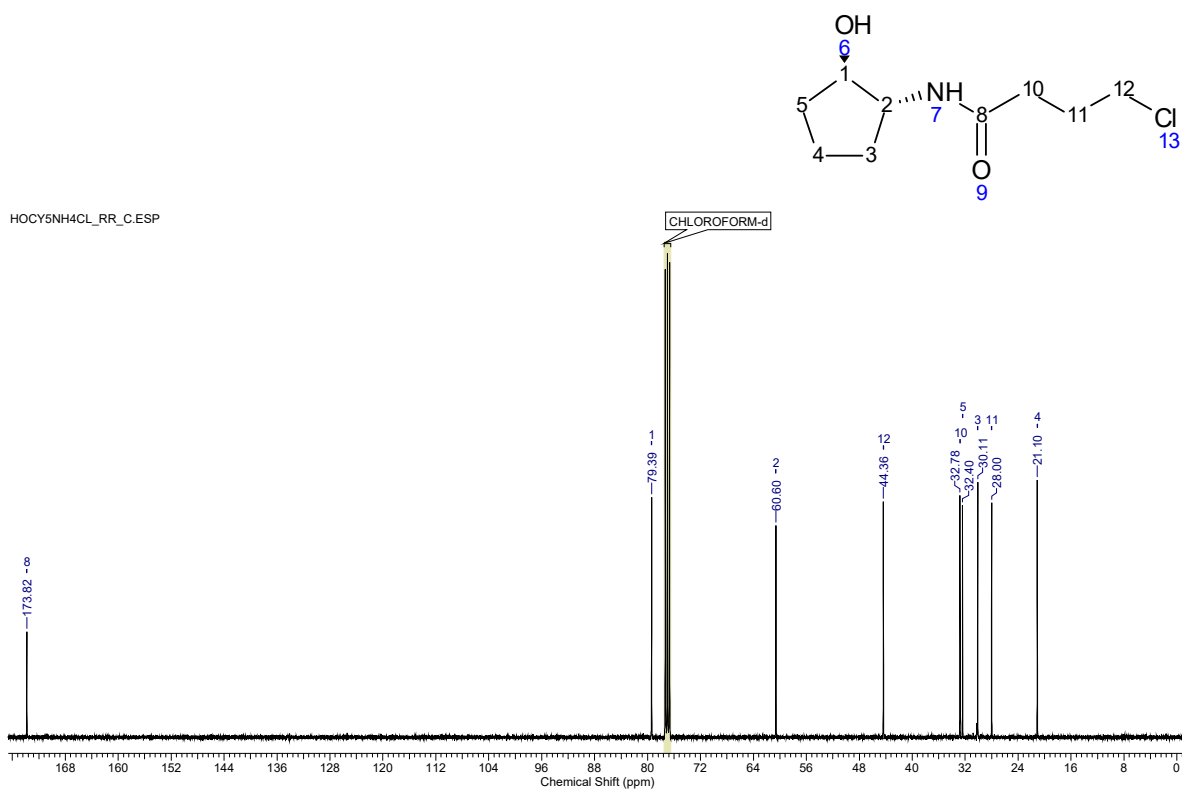
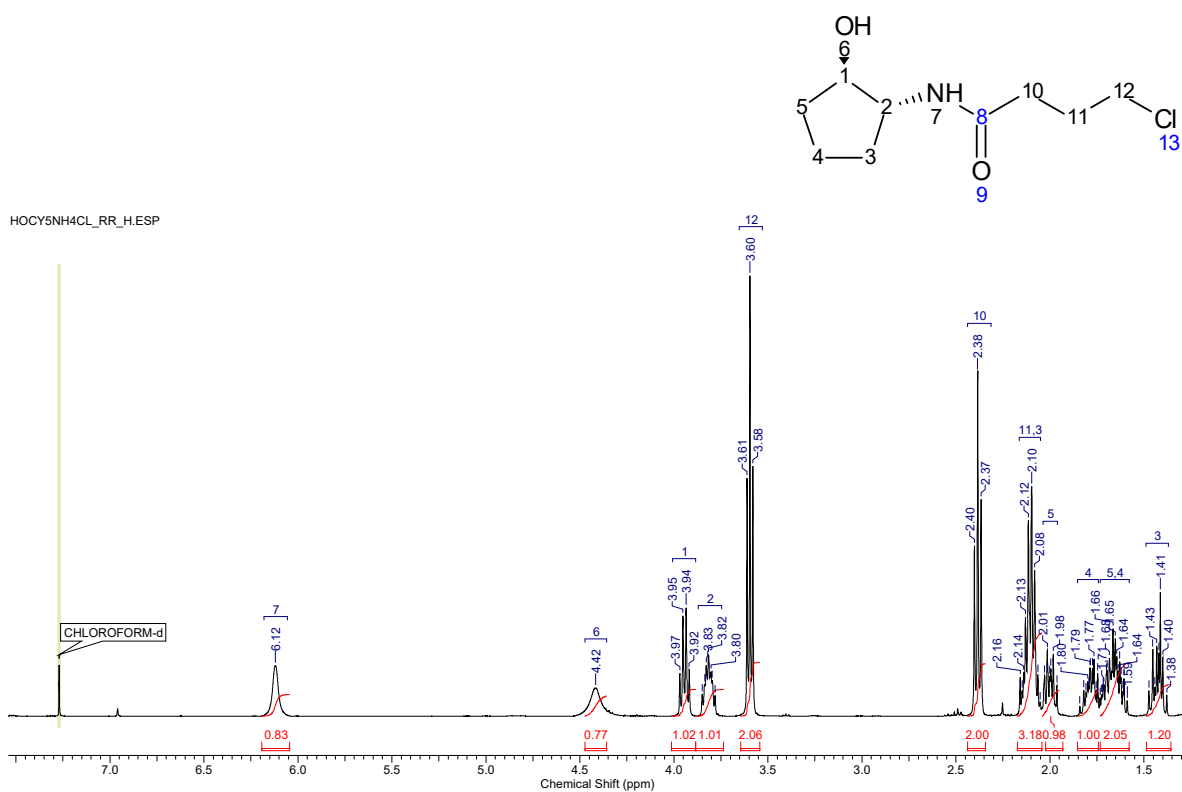
TBSOCY5NH4T4CIP\_SS\_C.ESP



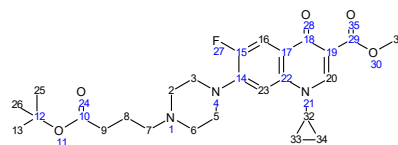
# 10.36 4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide 193



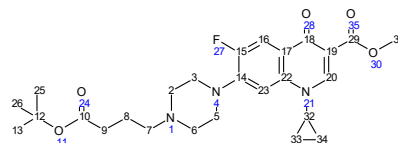
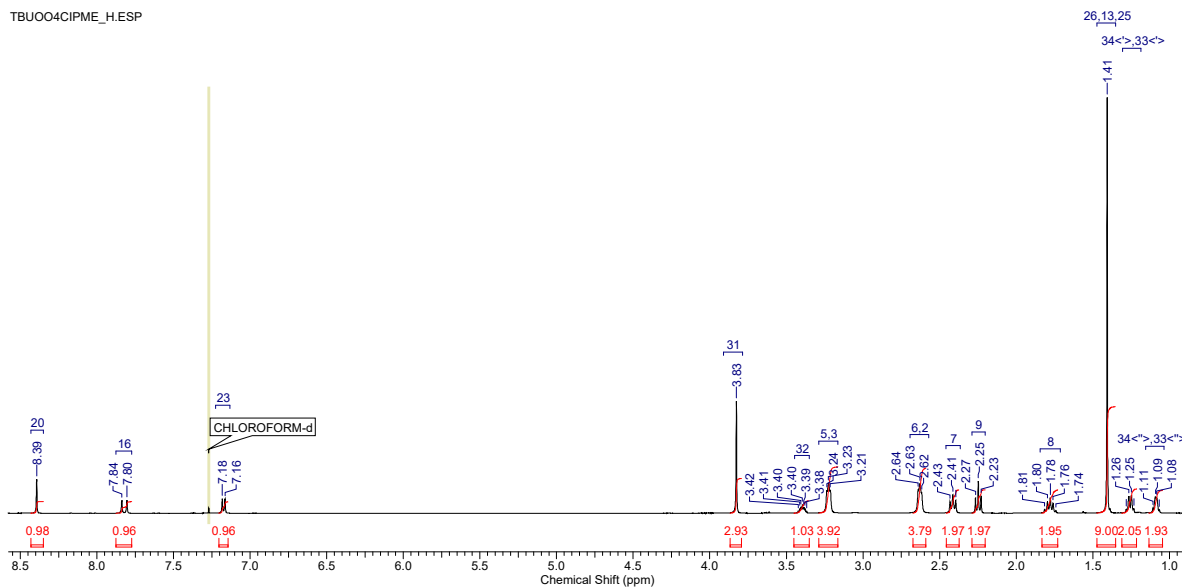
10.37 4-Chloro-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide 194



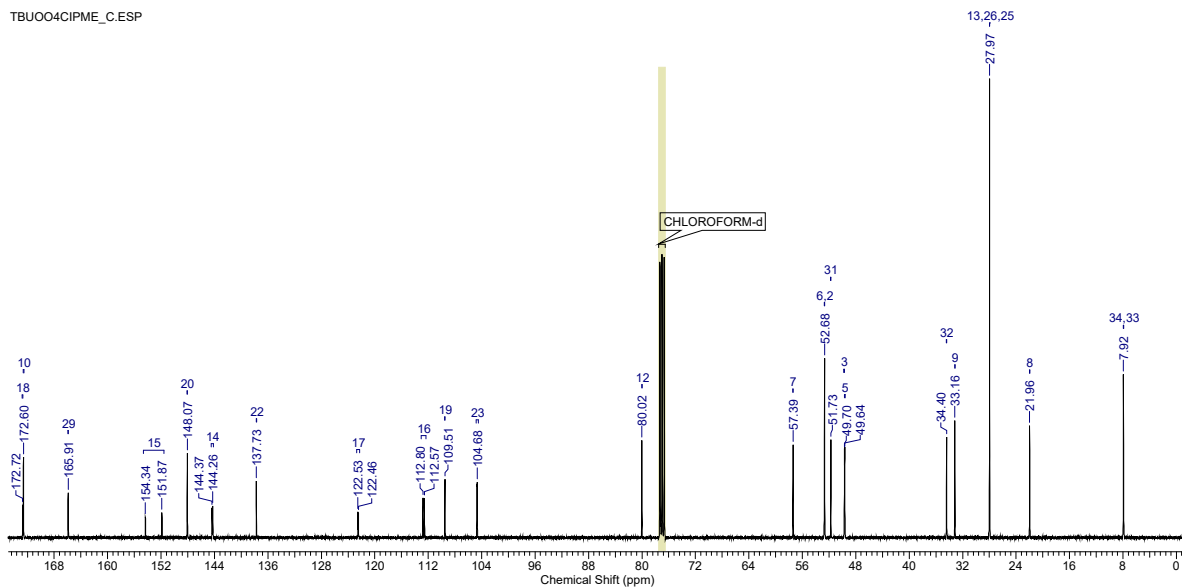
10.38 Methyl 7-(4-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 197



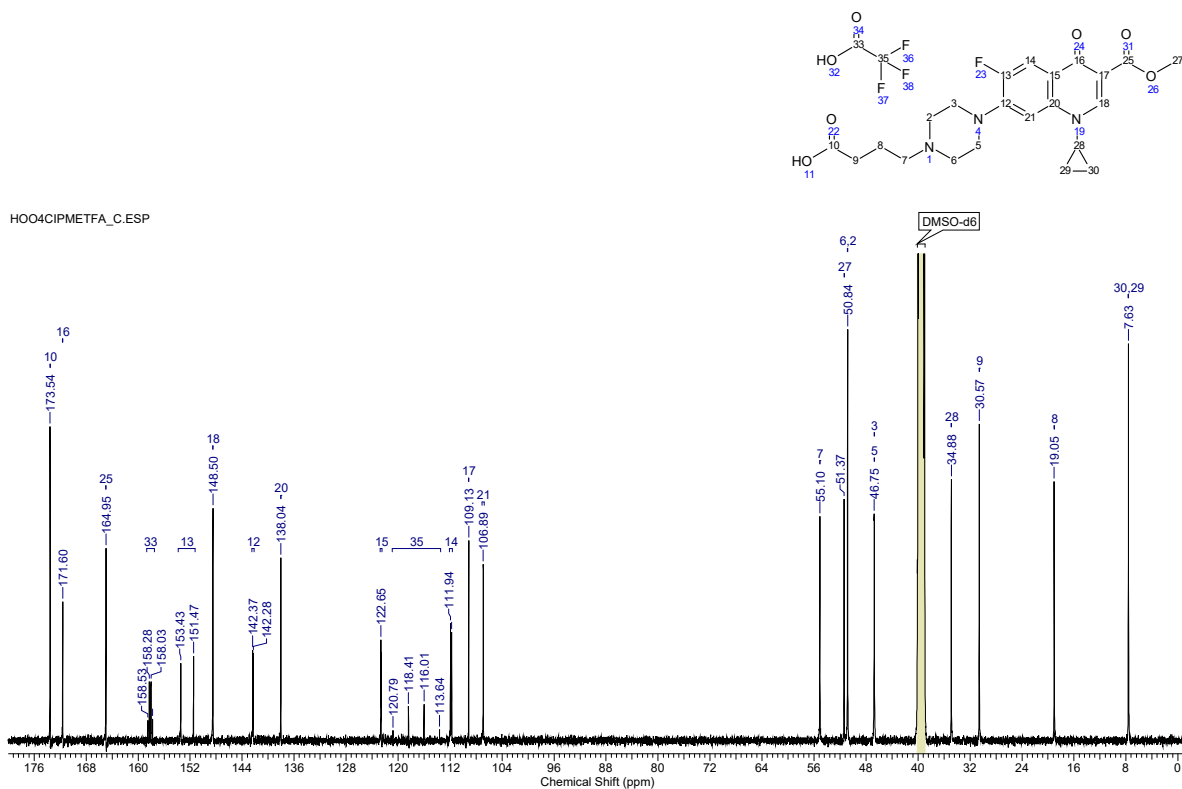
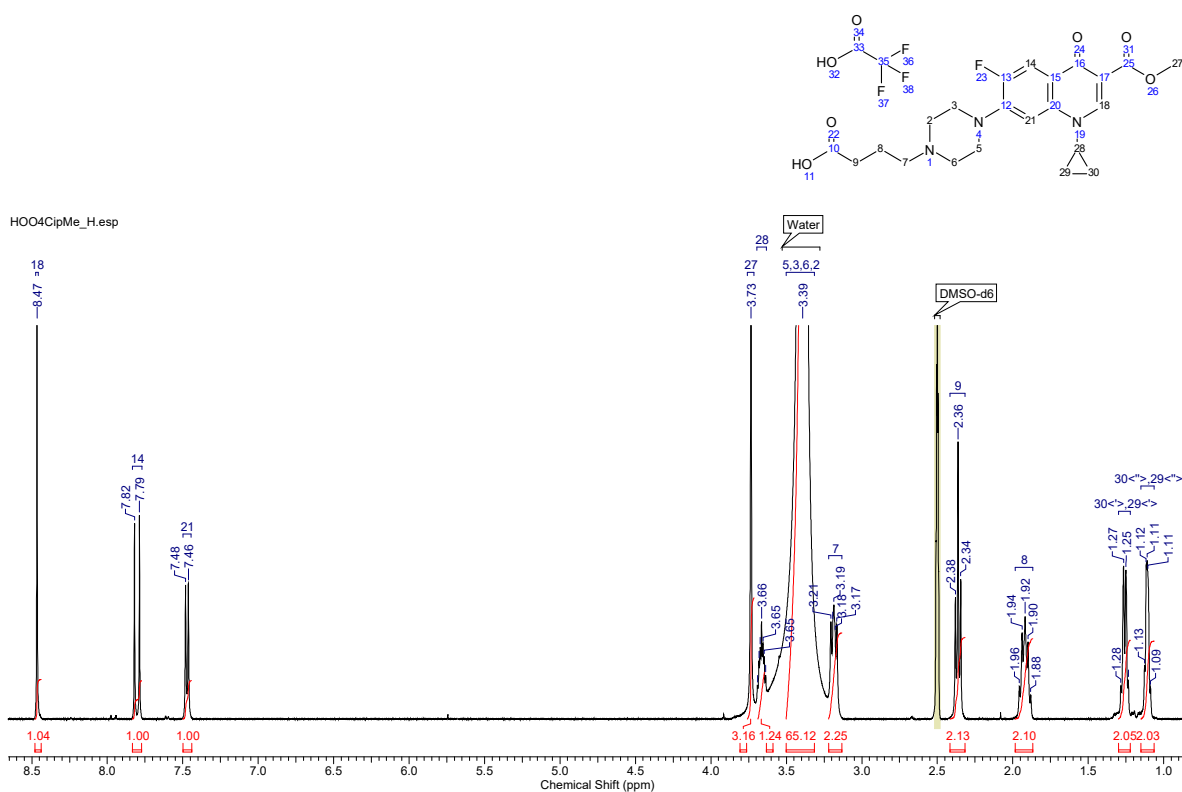
TBU004CIPME\_H.ESP



TBU004CIPME\_C.ESP

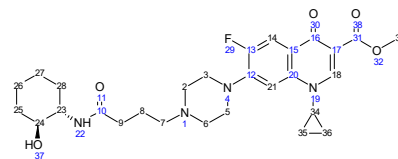


10.39 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt 198

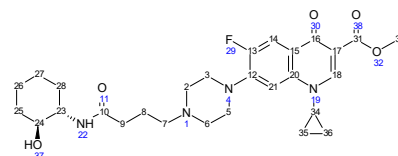
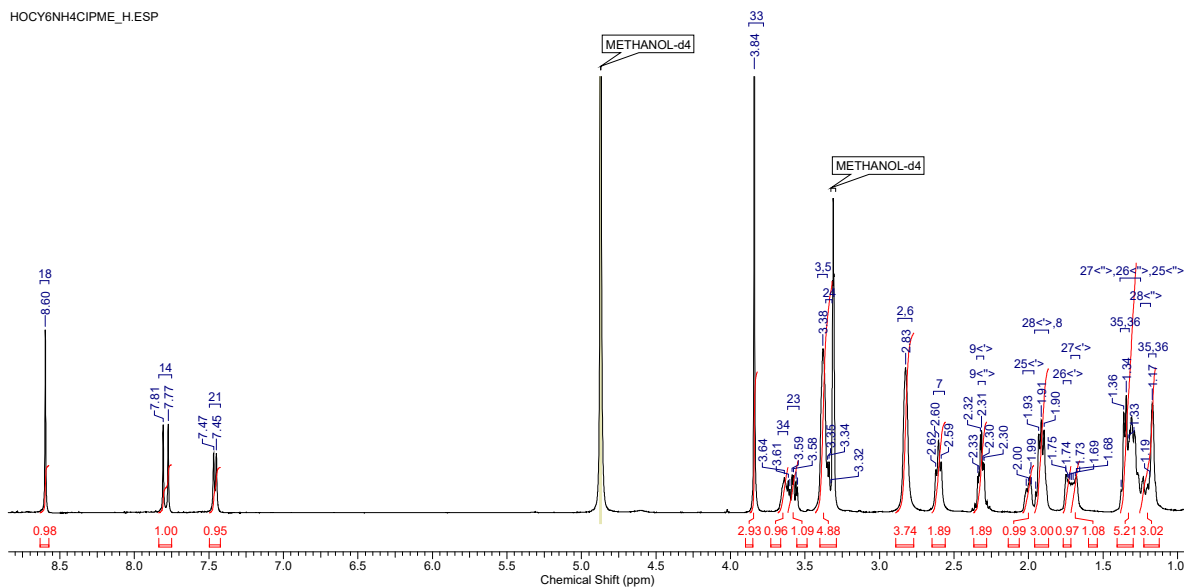




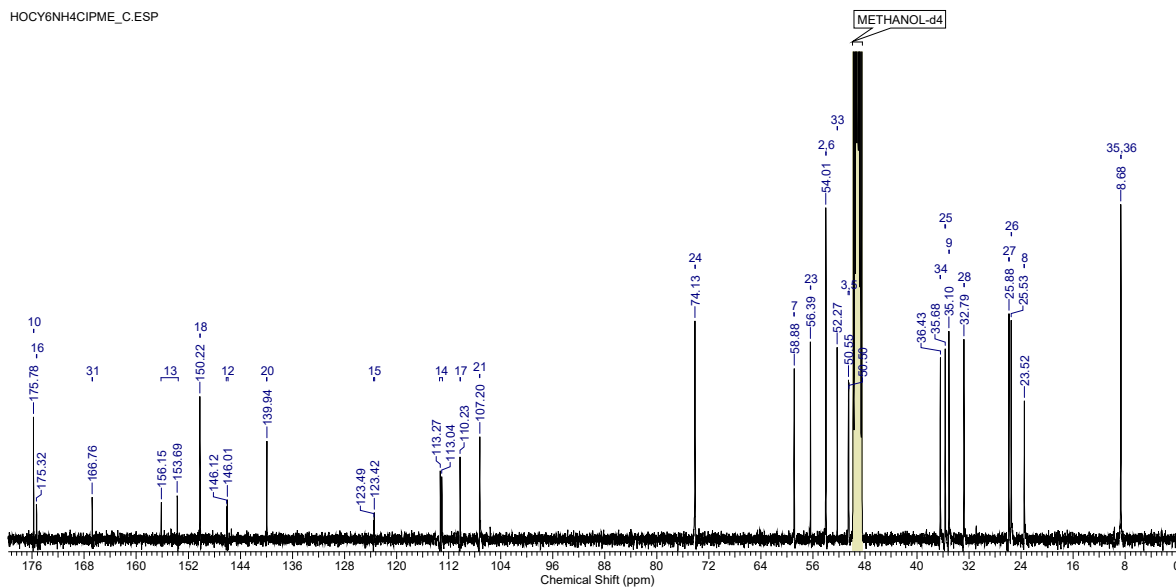
10.40 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201



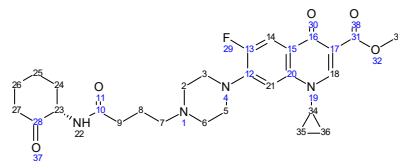
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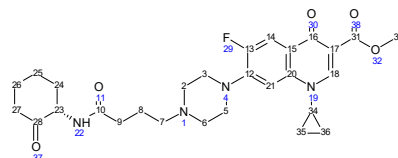
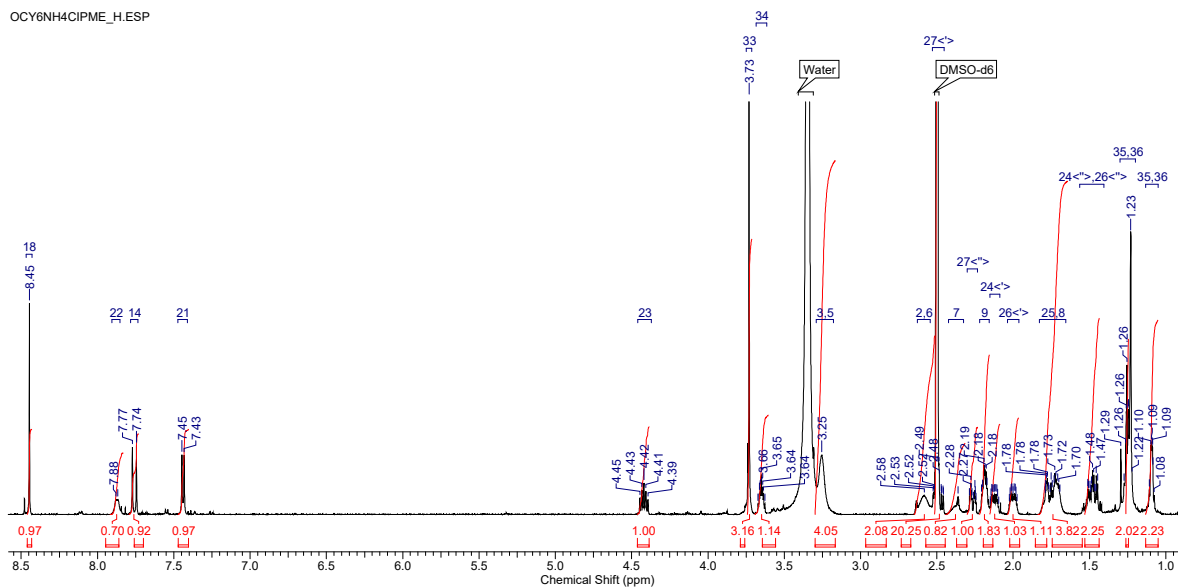
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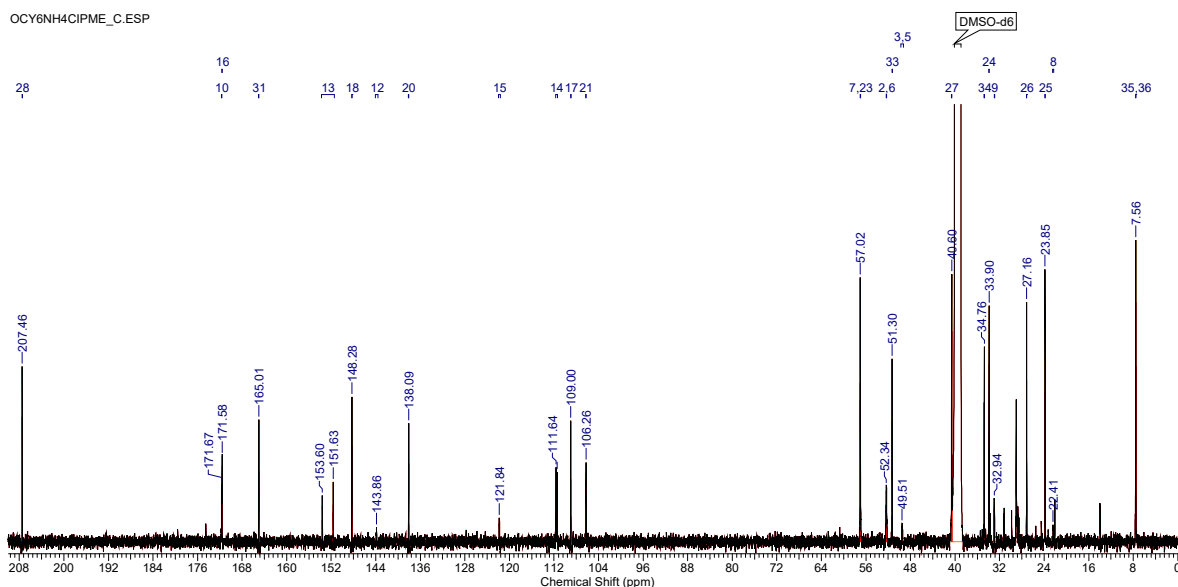
10.41 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 202



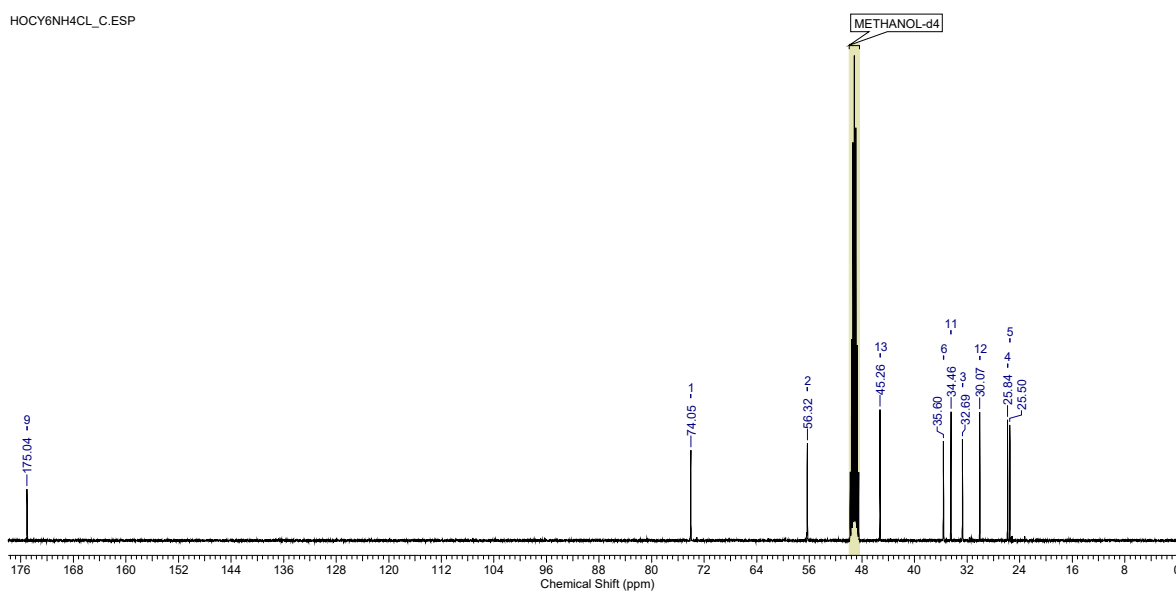
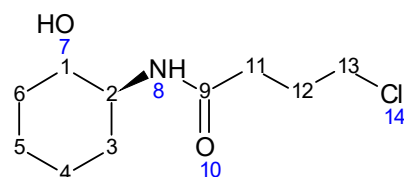
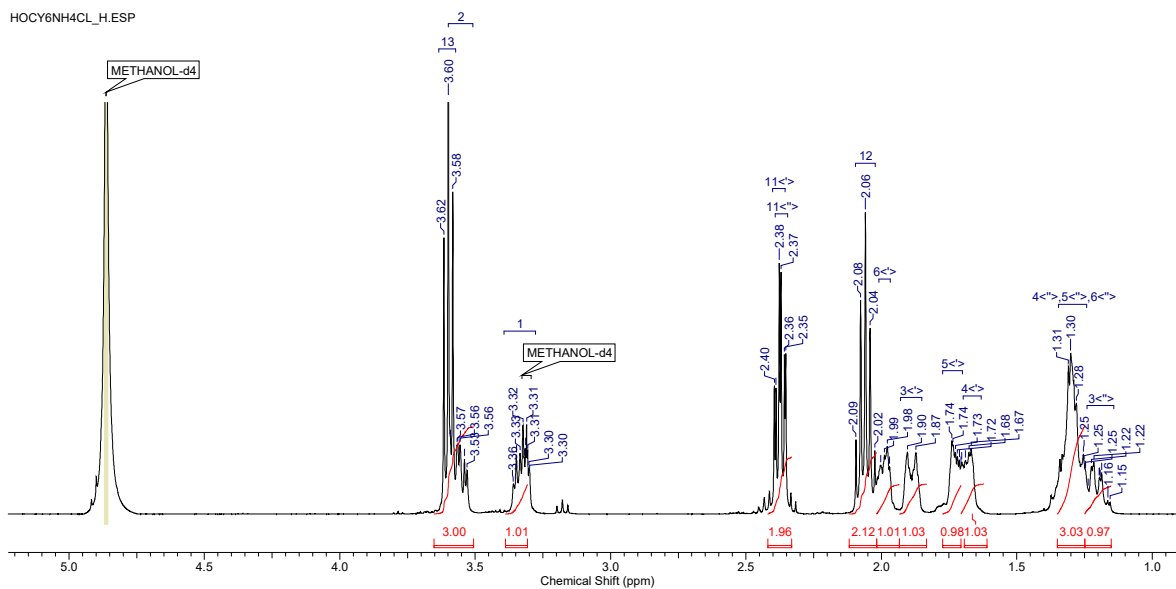
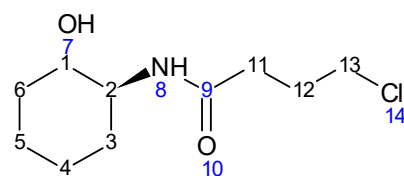
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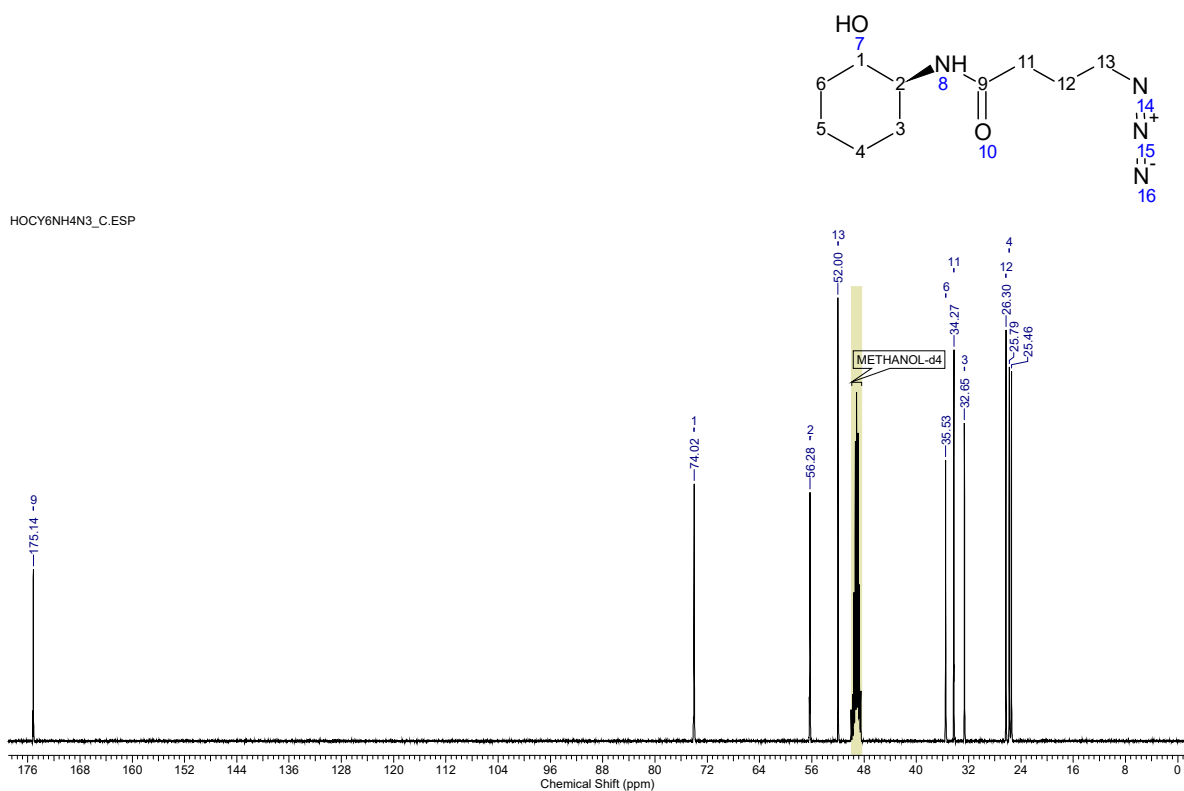
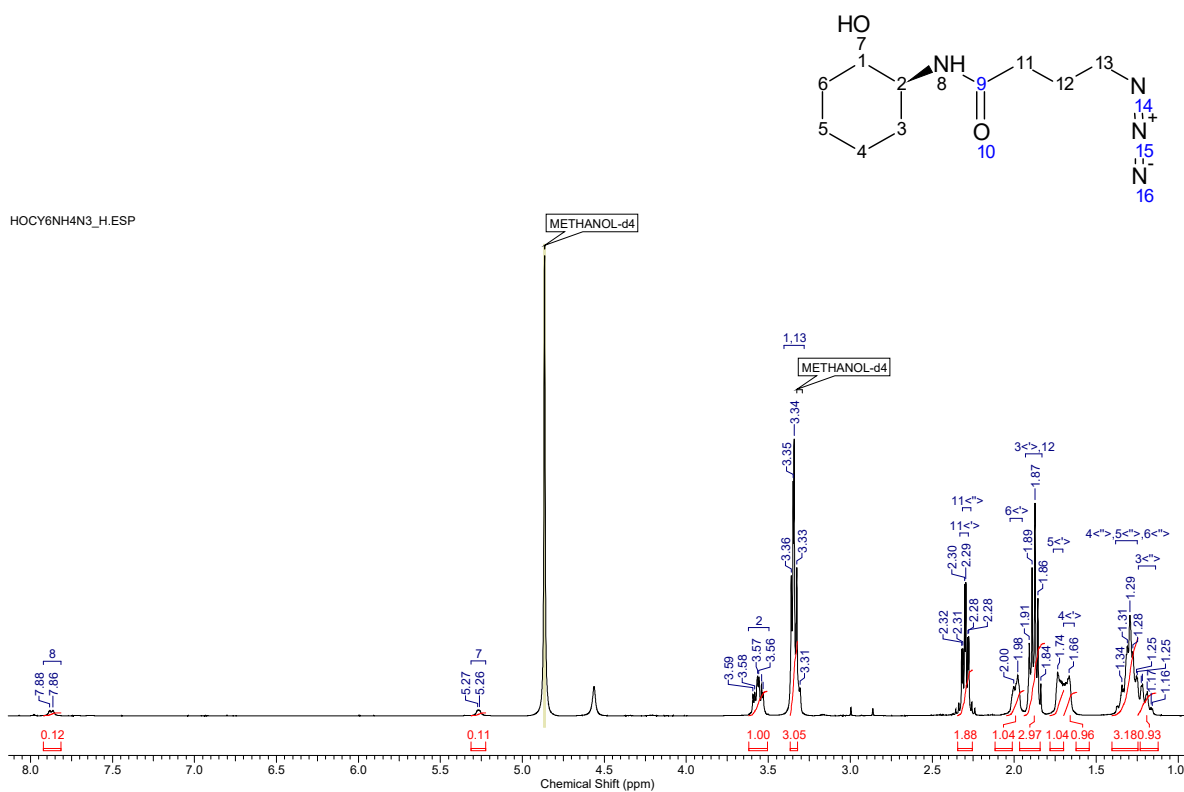
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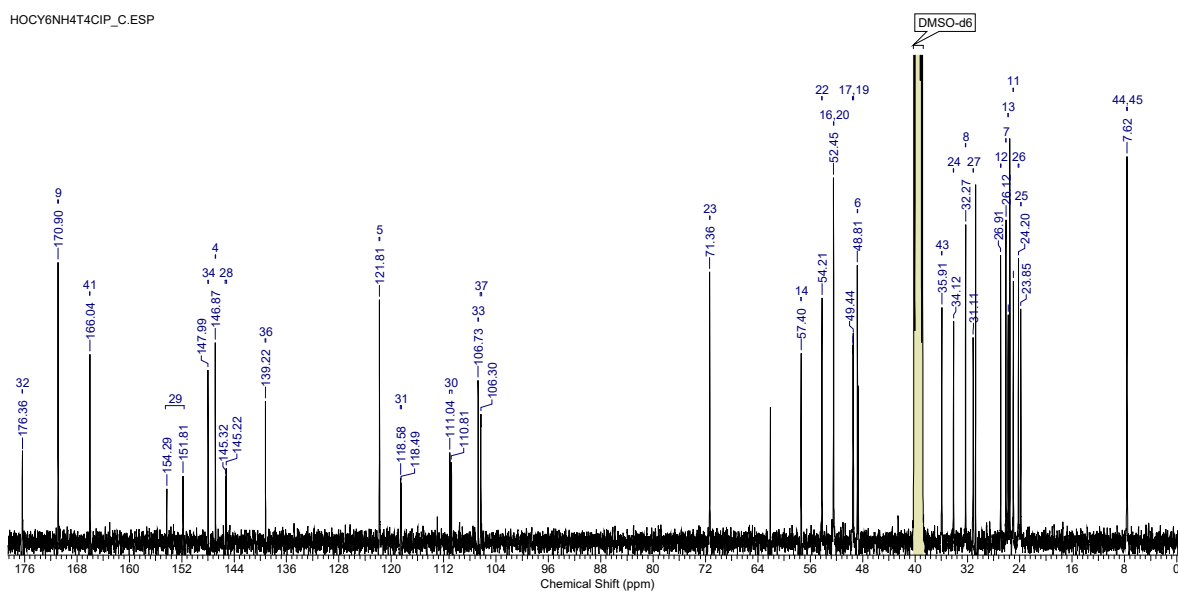
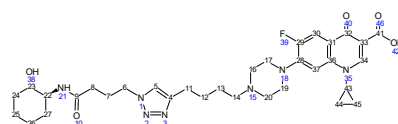
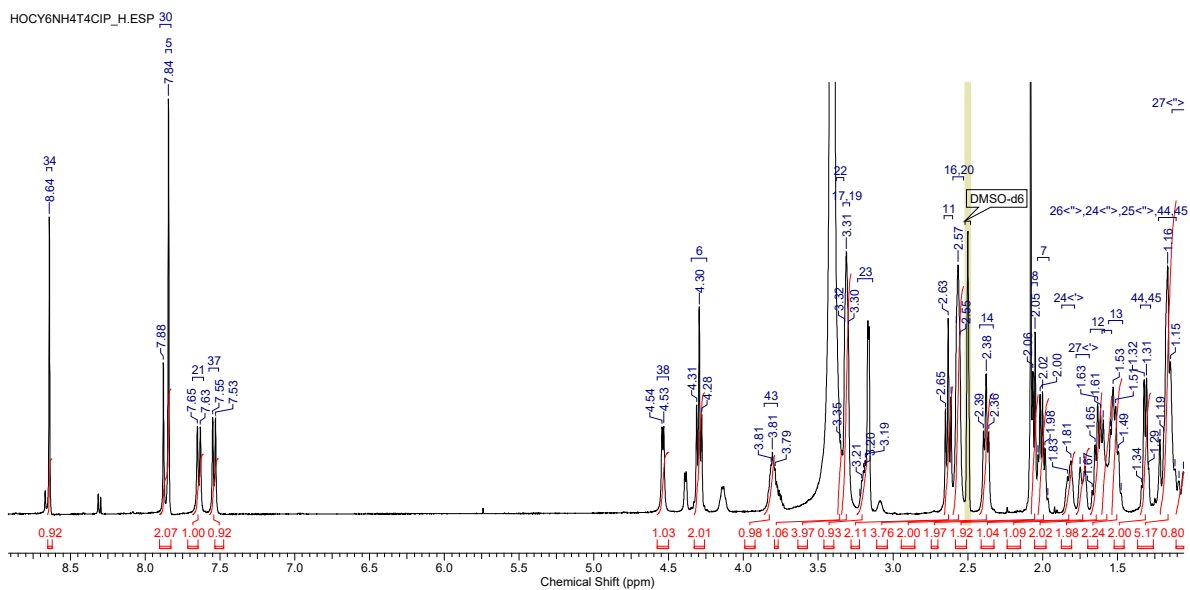
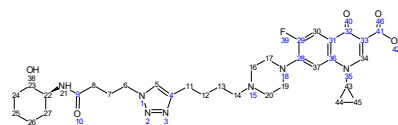
10.42 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 203

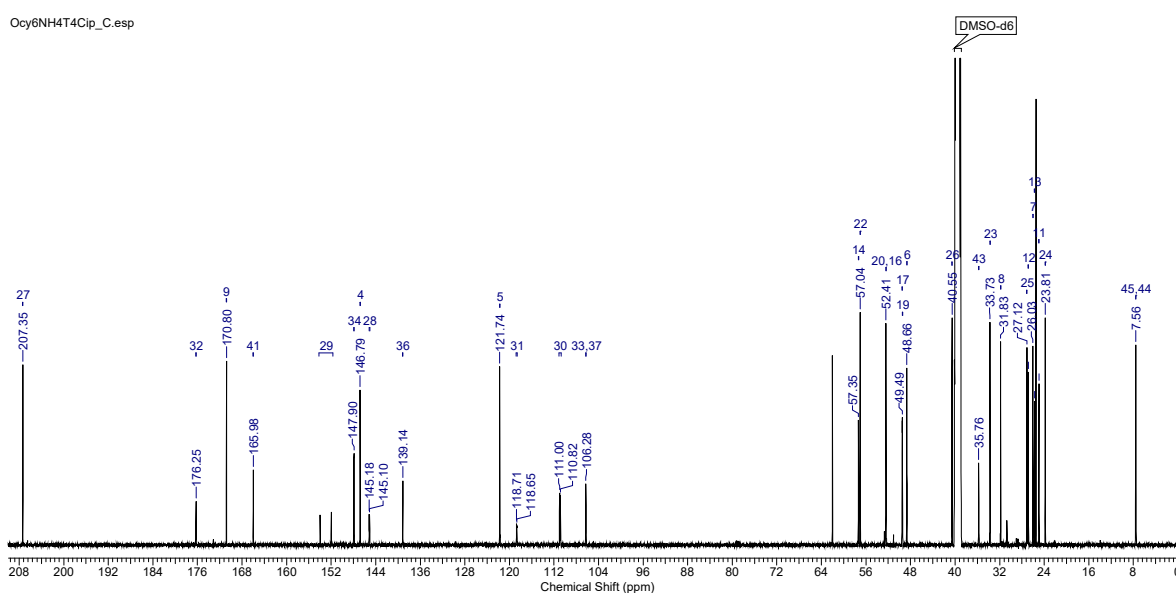
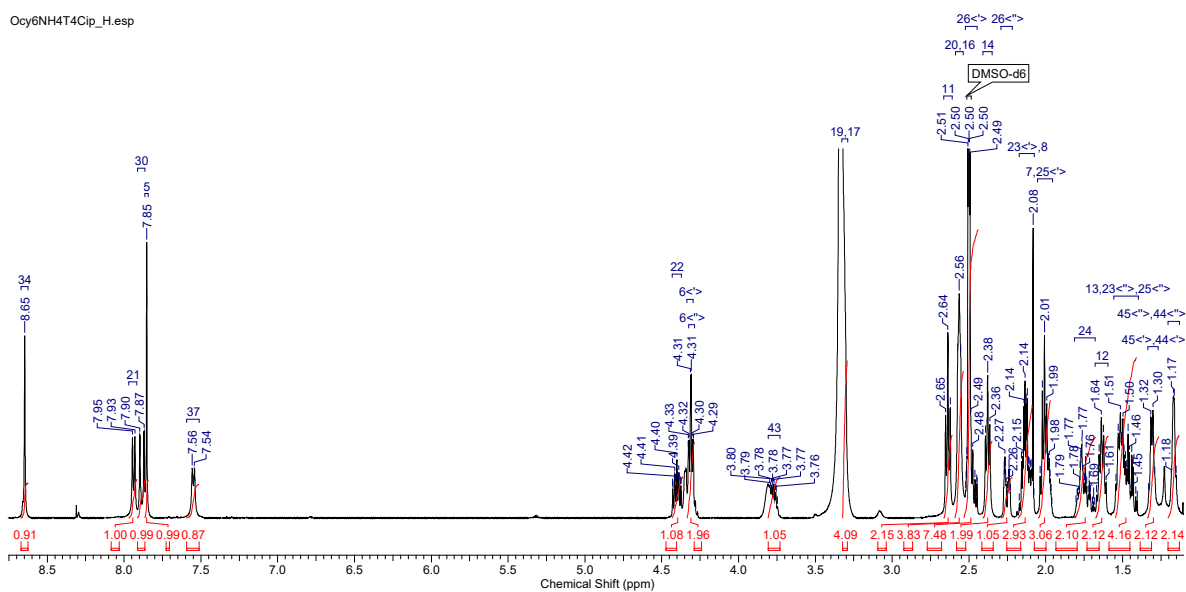


10.43 4-Azido-*N*-((*trans*-2-hydroxycyclohexyl)butanamide 204



10.44 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquino-  
line-3-carboxylic acid 205





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